Degradation of Epichlorohydrin and Halohydrins by Bacterial Cultures Isolated from Freshwater Sediment

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Three bacterial cultures, the Gram-negative strain AD1 and the Gram-positive strains AD2 and AD3, were isolated from freshwater sediment after enrichment with epichlorohydrin as sole carbon source. In batch cultures of strain AD1 and strain AD3, epichlorohydrin was rapidly degraded to 3-chloro-1,2-propanediol. Crude extracts of strain AD1 contained epoxide hydrolase activity towards epichlorohydrin, epibromohydrin, glycidol and propylene oxide as substrates. In contrast, strain AD2 did not actively convert epichlorohydrin but utilized 3-chloro-1,2-propanediol produced by slow chemical hydrolysis. No epichlorohydrin epoxide hydrolase was found in extracts of this organism. Crude extracts of strains AD1 and AD2 dehalogenated a number of mono- and dihalogenated alcohols and ketones, such as 1,3-dichloro-2-propanol, 3-chloro-1,2-propanediol, 1-chloro-2-propanol, 1,3-dibromo-2-propanol, chloroacetone and 1,3-dichloroacetone. Dehalogenation yielded epoxides as products. The results suggest that epichlorohydrin is converted by strain AD1 via 3-chloro-1,2-propanediol and glycidol by the action of an epoxide hydrolase and a dehalogenase, respectively. The same route for dehalogenation proceeds in strain AD2, which, however, is dependent on chemical hydrolysis of epichlorohydrin rather than enzymic conversion.

INTRODUCTION

During recent years, much insight has been obtained into the aerobic biodegradation of chlorinated aliphatic hydrocarbons (for a review see Vogel *et al.*, 1987). Relatively little is known about the microbial degradation of chlorinated aldehydes, alcohols and ethers, although several of them are important environmental pollutants (Ghisalba, 1983). Chloroethanol may serve as growth substrate for pure bacterial cultures and is degraded via chloroacetic acid (Stucki & Leisinger, 1983). There are indications that bromoethanol, epichlorohydrin and chloroethyl-ethers may be hydrolytically dehalogenated by bacterial dehalogenases (Janssen *et al.*, 1988; D. B. Janssen, A. J. van den Wijngaard & J. Prins, unpublished), although it is not clear whether the activities are involved in the utilization of these compounds. Enzymic formation of epoxides from halohydrins has been found in an organism that utilizes 2,3-dibromo-1-propanol (Castro & Bartnicki, 1968; Bartnicki & Castro, 1969).

Information is lacking on the bacterial degradation of halogenated epoxides, such as epichlorohydrin (3-chloro-1,2-epoxypropane) (Krijgheld & van der Gen, 1986). This compound is of great importance for chemical industries, where it is used for the production of glycerol and epoxy resins. Furthermore, it is an important starting material in the manufacture of a variety of surfactants, pharmaceuticals, insecticides, agricultural chemicals, coatings, adhesives, ion-exchange resins, solvents and plasticizers (Krijgheld & van der Gen, 1986).

Epichlorohydrin was classified as a black list pollutant by the Commission of European Communities in 1982. The black list contains 129 substances that are cause for great concern in the protection of aquatic life (Krijgheld & van der Gen, 1986). Because of the toxic properties of

epichlorohydrin and the lack of information on its biodegradation, we investigated whether bacterial utilization of this compound is possible, what organisms are involved and how degradation proceeds physiologically.

In this paper, three different bacterial cultures that are able to grow with epichlorohydrin as sole carbon source are described. The dehalogenation reactions were identified and a metabolic pathway for the biodegradation of epichlorohydrin is proposed.

METHODS

Organisms. Three different pure bacterial cultures, designated strain AD1, AD2 and AD3, were isolated from freshwater sediment that was polluted with chlorinated organics. Batch enrichments were carried out at 30 °C in MMY-medium (see below) supplemented with 1 mM-epichlorohydrin as carbon source. The inoculum for the enrichment was 10% (v/v) sediment. After several subcultivations in MMY-medium with 2.5 mM-epichlorohydrin, three pure isolates were obtained by repeated streakings on solid medium with 2.5 mM-epichlorohydrin as sole carbon source.

Spontaneous loss of the ability to grow on epichlorohydrin was observed for all three strains after two or more transfers on 0.8% nutrient broth agar. Therefore, the organisms were maintained on MMY-agar supplemented with epichlorohydrin. Epichlorohydrin-negative mutants of strain AD1 were isolated after several serial transfers in MMY-medium with 5 mM-glycidol as carbon source and maintained on glycidol-containing agar plates.

Media and growth conditions. The basal medium (MMY) that was used in all experiments contained (l⁻¹): 5.37 g Na₂HPO₄ · 12H₂O, 1.36 g KH₂PO₄, 0.5 g (NH₄)₂SO₄, 0.2 g MgSO₄ · 7H₂O, 5 ml trace metals solution (Janssen *et al.*, 1985) and 10 mg yeast extract (Difco laboratories). Cells were grown aerobically at 30 °C under rotary shaking. To prevent evaporation of substrates, cultures were grown in closed serum flasks, filled to one-third of their volume with medium. The flasks were made gas-tight with screw caps containing Viton septa, which made it possible to sample cultures by a syringe, without loss of substrates. Carbon sources were added up to 5 mM, if not stated otherwise, calculated as if the compounds added were completely dissolved in the water phase. All the halogenated compounds used were shown to be sterile. Serum flasks were closed immediately after addition of the compounds to prevent losses by evaporation. Inocula consisted of 5% (v/v) of late exponential epichlorohydrin-grown cultures.

Growth was followed turbidimetrically at 450 nm. Chloride levels in samples of culture fluids were determined with an ion-selective electrode (Orion type 94-17). Chloride levels in MMY-medium without halogenated substrate were less than 0.15 mM.

Preparation of crude extracts. Cells were harvested from late exponential cultures by centrifugation (15 min, 10000 g), washed once with 10 mM-Tris/H₂SO₄ buffer, pH 7.5, containing 1 mM-EDTA and 1 mM-β-mercaptoethanol, and suspended in this buffer at 0 °C. In this way a cell suspension of 30–50 mg dry wt ml⁻¹ was obtained. After ultrasonic disruption of the ice-cold cells, a crude extract (10–15 mg protein ml⁻¹) was obtained by centrifugation (30 min, 40000 g, 4 °C).

Activities in crude extracts. Dehalogenase activities were assayed at 30 °C in 50 mM-Tris/H₂SO₄ buffer, pH 7.5, containing 5 mM substrate and crude extract in a final volume of 3 ml. Assays contained 10–60 mU of enzyme ml⁻¹. At different times, all within 25 min, 0.5 ml samples were removed and assayed for halide levels by the colorimetric method of Bergmann & Sanik (1957). The detection limit of this method was 0.05 mM-chloride. Solutions of halogenated compounds used were prepared immediately before use. Chemical halide production was tested for all halogenated compounds used, and only found in controls with 1-bromo-2-propanol (initial velocity 2.5×10^{-3} mM min⁻¹), 1,3-dibromo-2-propanol (1.0×10^{-2} mM min⁻¹) and 1,3-dichloroacetone (1.2×10^{-2} mM min⁻¹). For the calculation of enzyme activities, chemical halide production was subtracted from total halide production. One unit of enzyme activity was defined as the amount of enzyme that catalyses the formation of 1 μmol halide min⁻¹.

Epoxide hydrolase activities were assayed at 30 °C in 50 mM-Tris/H₂SO₄ buffer, pH 7.5, containing 5 mM substrate and crude extract in a final volume of 3 ml. At different times within 25 min the epoxide concentration was analysed by gas chromatography. Chemical hydrolysis of the epoxides was negligible under these conditions. One unit of epoxide hydrolase was defined as the activity that catalyses the degradation of 1 μmol of epoxide min⁻¹.

For the identification of the products formed during conversion of halohydrins and epoxides in crude extracts, assays were run in a final volume of 0.5 ml in 1.5 ml vials, which were closed gas-tight with Teflon-lined caps. Samples were taken within 30 min and analysed by gas chromatography-mass spectrometry (GC-MS). In the case of less stable substrates, such as 1,3-dibromo-2-propanol, 1,3-dichloroacetone and 1-bromo-2-propanol, assay mixtures were incubated for 10 min, after which the products were analysed immediately.

Oxygen consumption by crude extracts was measured at 30 °C with a Clark-type oxygen electrode. Protein was determined by the method of Lowry using bovine serum albumin as the standard.

Gas chromatography. Epichlorohydrin was quantitatively determined by capillary gas chromatography. Equipment and the temperature program were as previously described (Janssen *et al.*, 1987). Culture samples (3 ml) were extracted with 1 ml diethylether containing 1 mM-trichloroethylene as internal standard and the ether layer was analysed by split injection of 1 µl samples in the gas chromatograph. Other epoxides and halohydrins were quantified similarly.

Diols were analysed by direct split injection of 0.02 µl aqueous samples. A CPSil5-CB column (25 m × 0.22 mm; Chrompack), was used with helium (110 kPa, vent flow 30 ml min⁻¹) as the carrier gas. Quantitative analysis of 3-chloro-1,2-propanediol was done in the same way, but the sample was mixed with an equal volume of water containing 1.0 mM-1,2-propanediol as the internal standard, before injection. Further conditions and detection were as for epichlorohydrin analysis.

Mass spectrometry. Aqueous solutions of 1,3-dichloro-2-propanol, 1-chloro-2-propanol, 3-chloro-1,2-propanediol, chloroacetone, 1,3-dichloroacetone, 1,3-dibromo-2-propanol, epibromohydrin, glycidol (3-hydroxy-1,2-epoxypropane), propylene oxide (1,2-epoxypropane), 1,2-propanediol, hydroxyacetone and epichlorohydrin were analysed by GC-MS. The instrument used was a Ribermag R10-10C, operated at an electron beam energy of 70 eV and a source temperature of 180 °C. Water samples (0.5 µl) containing 1–3 mM concentrations of compounds to be analysed were injected without extraction on a CPSil5-CB column (25 m × 0.35 mm) with helium as carrier gas (150 kPa). The temperature program used was 3 min at 50 °C followed by 10 °C min⁻¹ to 250 °C.

Chemicals. All organic compounds used were obtained from commercial sources (Janssen Chimica or Aldrich) and were at least 95% pure, as checked by gas chromatography and NMR spectroscopy. Exceptions were 1-chloro-2-propanol and 1-bromo-2-propanol, which contained about 20% 2-chloro-1-propanol and 2-bromo-1-propanol, respectively.

RESULTS

Characterization of strains AD1, AD2 and AD3

Three different bacterial cultures capable of growing with epichlorohydrin as sole source of carbon and energy were isolated from polluted freshwater sediment. The characteristics of strain AD1 (Table 1) suggested that this bacterium might belong to the genus *Pseudomonas* (Palleroni, 1984). Strain AD2 was tentatively identified as a strain of *Arthrobacter* (Keddie *et al.*, 1986) (Table 1). The bacterium appeared as coccoid cells, often in pairs, which in complex media underwent a marked change to irregular rods with post-fission outgrowths. Cells became coccoid in stationary cultures. Strain AD3 was a Gram-positive non-sporing curved rod, or coryneform bacterium (Jones & Collins, 1986) (Table 1). The organism often appeared as single cells but also formed pairs or short chains.

All three strains were able to grow on glycerol, glycidol or glucose, but not on propylene oxide, 1,2-propanediol, ethylene glycol, hydroxyacetone or methanol.

Utilization of halogenated compounds

The utilization of halogenated compounds other than epichlorohydrin by strains AD1, AD2 and AD3 was determined. Growth tests were done in liquid medium supplemented with different halogenated substrates at a concentration of 4 mM (Table 2).

The three strains were able to grow on epichlorohydrin and 3-chloro-1,2-propanediol. Strain AD2 and strain AD3 showed slow growth on 1,3-dichloro-2-propanol and were not able to degrade this compound completely within 6 d. In contrast, strain AD1 grew with a generation time of 3.3 h on 1,3-dichloro-2-propanol and utilized this compound completely. In all cases, growth was accompanied by halide release which strongly exceeded uninoculated controls. No growth was observed with all three strains on chloroacetic acid, 3-chloropropionic acid, 2-chloropropionic acid, 1,3-dichloropropane, 1,3-dichloropropene, 3-chloropropene, 1-chloro-2-propanol, chloroacetone, 1,3-dichloroacetone, 2-chloro-3-butanone, 1,2-dichloropropane, 2-chloroethanol, 2-bromoethanol and 1,3-dibromo-2-propanol.

Degradation of epichlorohydrin in batch cultures

Growth of strains AD1 and AD3 on 5 mM-epichlorohydrin was followed in batch cultures (Fig. 1a, b). Almost immediately after inoculation, there was a fast decrease of the epichlorohydrin concentration resulting in a complete depletion of the compound in the lag phase. No significant chloride production occurred during this phase of rapid epichlorohydrin

Table 1. *Some properties of the epichlorohydrin-utilizing bacterial strains AD1, AD2 and AD3*

Property	Strain AD1	Strain AD2	Strain AD3
Gram reaction	Negative	Positive	Positive
Acid-fast staining	ND	—	—
Oxidase	+	+	+
Catalase	+	+	+
Nitrate reduction	+	—	—
Fermentation	—	—	—
Endospores formed	—	—	—
Flagellation	+*	—	—
Growth at 37 °C	ND	—	+
Morphology†	Rod	Irregular	Curved rod
Motility	+	—	—
Pigmentation	None	Cream-coloured	Yellow/green-coloured
Colony morphology	Circular, convex, entire, translucent	Circular, convex, lobate, opaque	Circular, convex, entire, opaque
Length (µm)	2.0–3.0	0.7–1.5	0.7–0.9
Width (µm)	0.8–1.0	0.6–0.9	0.5–0.7

ND, Not determined.

* One polar flagellum visible by electron microscopy.

† Cells were grown in MMY-medium containing 5 mM-glucose.

Table 2. *Utilization of halogenated compounds by strains AD1, AD2 and AD3*

Carbon sources were added to a final concentration of 4 mM. Precultures were grown on 4 mM-epichlorohydrin.

Carbon source	Generation time (h)		Halide produced (mM)*			
	AD1	AD3	AD1	AD2	AD3	Sterile control
Epichlorohydrin	5.5	14.0	4.2	3.4	4.2	0.2
1,3-Dichloro-2-propanol	3.3	—	7.9	4.7	3.2	1.2
3-Chloro-1,2-propanediol	3.1	10.5	4.2	3.9	3.6	0.3

—, Non-exponential growth.

* Halide levels were measured after 6 d cultivation.

turnover. The conversion of epichlorohydrin was due to a high enzymic activity since its concentration decreased much more slowly in sterile controls (Fig. 1*d*).

The accumulating halogenated compound in cultures of AD1 and AD3 was identified by GC-MS as 3-chloro-1,2-propanediol, which is in agreement with the absence of chloride production during epichlorohydrin degradation. The halohydrin was also identified in the culture fluids of a mutant of strain AD1 lacking dehalogenase activity (see below). 3-Chloro-1,2-propanediol was stable in sterile medium, showing no significant chloride release (Table 2). Both strains AD1 and AD3 were able to utilize this compound as a carbon source and actively degraded epichlorohydrin. The growth rates on epichlorohydrin (Table 2) may thus be determined by the rate of metabolism of 3-chloro-1,2-propanediol. The growth of strain AD1 was much faster than that of strain AD3 for the three halogenated compounds mentioned in Table 2 and therefore we chose strain AD1 for further study.

The decrease of epichlorohydrin concentration in a batch culture of strain AD2 (Fig. 1*c*) proceeded at a rate similar to the sterile control (Fig. 1*d*). Epichlorohydrin was slowly converted with a half-life of about 70 h, in agreement with earlier findings of Ross (1950). This suggests that in contrast to strains AD1 and AD3, strain AD2 was not capable of active degradation of epichlorohydrin but merely utilized the product of chemical hydrolysis, 3-chloro-1,2-propanediol. Also, strain AD2 showed non-exponential growth with 3-chloro-1,2-propanediol and 1,3-dichloro-2-propanol, the latter compound being only partially degraded.

Fig. 1. Batch cultures on 5 mM-epichlorohydrin of strains AD1 (a), AD3 (b) and AD2 (c). (d) Sterile control. ○, OD₄₅₀; ▲, chloride; ■, epichlorohydrin; □, 3-chloro-1,2-propanediol; ●, pH.

Because of the inability of strain AD2 to actively degrade epichlorohydrin to 3-chloro-1,2-propanediol, we decided to compare the properties of this strain in more detail with the epichlorohydrin-utilizing strain AD1.

Degradation of epoxides by crude extracts

The rapid transient accumulation of 3-chloro-1,2-propanediol observed in cultures of strain AD1 suggests that this bacterium produces an epoxide hydrolase activity toward epichlorohydrin. Therefore, crude extracts were prepared from epichlorohydrin-grown cells and incubated with several epoxides (Table 3). The rate of epoxide degradation was highest with propylene oxide, but epibromohydrin and epichlorohydrin were also rapidly converted.

The epoxidase activity was present in extracts of citrate- or glucose-grown cultures of strain AD1 at levels of 59 and 137 mU (mg protein)⁻¹, respectively, which is lower than the activity in cells from epichlorohydrin-grown cultures (Table 3). Glycidol-grown cells of strain AD1 contained 817 mU (mg protein)⁻¹ of epoxide-degrading activity, showing that the formation of this activity is inducible.

Table 3. *Epoxide hydrolase activities towards various substrates in crude extracts of strains AD1 and AD2 grown on epichlorohydrin*

Strain	Substrate	Specific activity [mU (mg protein) ⁻¹]
AD1	Epichlorohydrin	550
AD1	Epibromohydrin	854
AD1	Propylene oxide	3000
AD1	Glycidol	107
AD2	Epichlorohydrin	<10

Table 4. *Dehalogenating activities towards various substrates in crude extracts of strains AD1, AD1M12 and AD2 grown on epichlorohydrin or on citrate*

Substrate	Specific activity [mU (mg protein) ⁻¹]				
	Epichlorohydrin		Citrate		
	AD1	AD2	AD1	AD1M12*	AD2
1,3-Dichloro-2-propanol	287	870	22	<4	38
1-Chloro-2-propanol	260	178	13	<4	8
3-Chloro-1,2-propanediol	20	167	—	—	—
1,3-Dibromo-2-propanol	5022	117000	297	<4	9268
1-Bromo-2-propanol	1109	30070	63	<4	1220
1,3-Dichloroacetone	337	2159	24	<4	141
Chloroacetone	87	113	—	—	—
Epichlorohydrin	<10	<4	—	—	—
Chloroacetic acid	<10	<4	—	—	—
2-Bromoethanol	109	691	—	—	—

—, Not measured.

* Epichlorohydrin-negative mutant of strain AD1 (see text).

Crude extracts of strain AD2 grown on epichlorohydrin, in contrast, did not actively convert epichlorohydrin (Table 3). This conversion was also not found in batch cultures of strain AD2 grown on epichlorohydrin (Fig. 1), indicating that strain AD2 does not produce an epichlorohydrin epoxide hydrolase.

Dehalogenations catalysed by crude extracts of strains AD1 and AD2

The dehalogenation capacities of strains AD1 and AD2 were investigated by incubating various halogenated compounds with crude extracts prepared from cells of late exponential cultures grown on 5 mM-epichlorohydrin or on 5 mM-citrate (Table 4). Strain AD1 showed very little dehalogenating activity and strain AD2 showed no activity at all with epichlorohydrin. Halide release in crude extracts of strains AD1 and AD2 was observed with haloalcohols such as 1,3-dichloro-2-propanol, 1-chloro-2-propanol, 3-chloro-1,2-propanediol, 1,3-dibromo-2-propanol and 1-bromo-2-propanol. Halide production was also found with chloroacetone and 1,3-dichloroacetone. No activity of strains AD1 and AD2 was found with 2-chloropropionic acid, 1,3-dichloropropane, 1,3-dichloropropene, 1-chloropropane, 2-chloropropane, 1,2-dichloropropane, 1-bromo-3-propanol, 2-chloro-1-propanol, 2-bromo-1-propanol, 1,2-dichloroethane, 1-chlorobutane and 2-chloro-3-butanone. The dehalogenating systems had different relative activities for the halogenated compounds tested, which indicates that the enzymes involved probably are not identical.

The dehalogenating activities were due to enzymic conversion since heat treatment of crude extracts of strains AD1 and AD2 (5 min at 100 °C) resulted in complete disappearance of activity with 1,3-dichloro-2-propanol, 1,3-dibromo-2-propanol and 1,3-dichloroacetone as substrates. None of the dehalogenating enzymes needed oxygen or cofactors for activity. During

Table 5. *Products formed in crude extracts of strains AD1 and AD2 grown on epichlorohydrin*

Substrate	Products found	
	Strain AD1	Strain AD2
1,3-Dichloro-2-propanol	3-Chloro-1,2-propanediol	Epichlorohydrin
1-Chloro-2-propanol	1,2-Propanediol	Propylene oxide
3-Chloro-1,2-propanediol	*	Glycidol
Epichlorohydrin	3-Chloro-1,2-propanediol	No conversion
Chloroacetone	Hydroxyacetone	Hydroxyacetone
1,3-Dibromo-2-propanol	Epibromohydrin, 3-bromo-1,2-propanediol	Epibromohydrin
Epibromohydrin	3-Bromo-1,2-propanediol	No conversion
1-Bromo-2-propanol	Propylene oxide, 1,2-propanediol	Propylene oxide
Propylene oxide	1,2-Propanediol	No conversion

* No product found by GC-MS.

conversion of 2 mM-1,3-dichloro-2-propanol by crude extracts of strains AD1 and AD2 in a biological oxygen monitor, no oxygen consumption was detectable.

Crude extracts of strain AD1 grown on citrate possessed 5–10% activity for halohydrins compared to extract from epichlorohydrin-grown cells, indicating that the dehalogenating enzyme is largely inducible by epichlorohydrin. This appeared to be the same with strain AD2 (Table 4).

Isolation of an epichlorohydrin-negative mutant of strain AD1

To obtain further insight into the enzymic conversion of epichlorohydrin, a mutant of strain AD1 was isolated after several serial transfers in MMY-medium with 5 mM-glycidol (see Methods). The mutant, designated strain AD1M12, had lost both its capacity to grow on epichlorohydrin and 3-chloro-1,2-propanediol but not on glycidol. Furthermore, no dehalogenating activity was detected in cells grown on citrate (Table 4). This was due to mutational loss of the dehalogenating enzyme, since strain AD1 grown on citrate still possessed activity for halohydrins.

To discriminate between a possible epoxide hydrolase and the dehalogenating enzyme of strain AD1, 30 mM-epichlorohydrin was added to a 3 mM-glycidol-grown culture of mutant AD1M12. After 16 h no epichlorohydrin was left and there was also no increase in chloride concentration. The accumulated intermediate was analysed by GC-MS and found to be 3-chloro-1,2-propanediol. Since the formation of this diol and the depletion of epichlorohydrin were much faster than could possibly be accomplished by chemical hydrolysis (Fig. 1*d*; Ross, 1950), it was concluded that uptake was not affected and that conversion of epichlorohydrin to 3-chloro-1,2-propanediol was due to an epoxide hydrolase, which is different from the dehalogenating enzyme and still present in the mutant.

Products formed in crude extracts

The products formed during incubation of halohydrins and epoxides with crude extracts prepared from cells of strains AD1 and AD2 grown on epichlorohydrin were analysed by GC-MS. Extracts of strain AD1 converted epichlorohydrin, epibromohydrin and propylene oxide to their corresponding diols (Table 5), again showing the presence of an epoxide hydrolase activity involved in epichlorohydrin metabolism (Fig. 2).

Extracts of strain AD1 as well of strain AD2 catalysed dehalogenation of halohydrins (Table 4). The mode of action of the dehalogenating systems was indicated by the conversion of 1,3-dibromo-2-propanol to epibromohydrin and 1-bromo-2-propanol to propylene oxide (Table 5). Dehalogenation thus occurred with concomitant production of an epoxide, indicating the

Fig. 2. Proposed routes for the degradation of epichlorohydrin. Strain AD1: epoxide hydrolase (1), halohydrin dehalogenase (2) and epoxide hydrolase (3). Strain AD2: chemical conversion (1), halohydrin dehalogenase (2) and chemical conversion (3).

action of an epoxide-forming halohydrin dehalogenase (Fig. 2). Prolonged incubation (30 min) of extracts of strain AD1 with these bromohydrins yielded also 3-bromo-1,2-propanediol and 1,2-propanediol.

With crude extract of strain AD2, epoxides were identified as the sole products formed from 1,3-dichloro-2-propanol, 3-chloro-1,2-propanediol and 1-chloro-2-propanol.

Upon incubation with extract from strain AD1, however, the corresponding diols rather than epoxides were produced from 1,3-dichloro-2-propanol and 1-chloro-2-propanol. Under the assay conditions used, the rates of epoxide hydrolysis of epichlorohydrin and propylene oxide are much higher than the rate of formation of these compounds from vicinal chlorohydrins by dehalogenase activity (Tables 3 and 4). Only when bromohydrins were used as the substrates for extract of strain AD1 were epoxides detected. The activity of the dehalogenase for these bromohydrins was much higher than for chlorohydrins (Table 4). With extract of strain AD1, no epoxide product was found with 3-chloro-1,2-propanediol, again probably due to the interference of the epoxide hydrolase (Table 3).

DISCUSSION

The results presented in this paper show that epichlorohydrin and several related halohydrins may serve as sole carbon source for bacterial growth. Although growth on vicinal halohydrins has been found previously (Castro & Bartnicki, 1968; Stucki & Leisinger, 1983; Janssen *et al.*, 1985), growth on epichlorohydrin had not been reported (Krijgheld & van der Gen, 1986).

Two patterns of epichlorohydrin degradation were observed in batch cultures. Strain AD1, a *Pseudomonas*-like organism, and the coryneform bacterium strain AD3, rapidly degraded epichlorohydrin in the lag phase of growth to 3-chloro-1,2-propanediol and then utilized this compound. Strain AD2, on the other hand, was not capable of active utilization of epichlorohydrin but only metabolized 3-chloro-1,2-propanediol produced by chemical degradation.

In strain AD1, epichlorohydrin degradation apparently started with an epoxide hydrolase reaction producing a diol rather than with a hydrolytic dehalogenation leading to glycidol (Fig. 2). With crude extracts and purified dehalogenase of *Xanthobacter autotrophicus* GJ10, which is

able to utilize 1,2-dichloroethane (Janssen *et al.*, 1985; Keuning *et al.*, 1985), we have found chloride release with epichlorohydrin as the substrate at 12% of the rate found with 1,2-dichloroethane. Thus, as an alternative, conversion of epichlorohydrin to glycidol as the first step of epichlorohydrin degradation would theoretically be possible, but this route does not appear to be of importance in the organisms described here.

The epoxide hydrolase of strain AD1 probably has a broad substrate range, catalysing the hydrolysis of epichlorohydrin, epibromohydrin, glycidol and propylene oxide. No conversion of these compounds was found in extracts of strain AD2 and the lack of active epichlorohydrin utilization in this strain thus appeared to be caused by the absence of epoxide hydrolase activity. Thus growth of strain AD2 with epichlorohydrin as carbon source will be limited by the rate of chemical hydrolysis of epichlorohydrin. Ross (1950) examined the kinetics of epoxide-ring opening reactions and found a rate constant of 0.019 h^{-1} for epichlorohydrin hydrolysis at 37°C and neutral pH, which is of the same order of magnitude as the half-life of 70 h at 30°C found here.

Hydrolysis of the epoxide ring as a first step in epichlorohydrin conversion has also been found in rats (Fakhouri & Jones, 1979; Jones & Fakhouri, 1979). As an alternative, epichlorohydrin may be metabolized via glutathione conjugation, which finally yields 2,3-dihydroxy-1-*S*-propylcysteine and its *N*-acetyl derivative, the major metabolites found in urine (Fakhouri & Jones, 1979; Jones & Fakhouri, 1979).

Information about bacterial epoxide hydrolase activity is scarce. One of the few descriptions of this enzyme concerns a propylene-oxide-utilizing *Nocardia* which produces an inducible epoxidase activity that catalyses the conversion of propylene oxide to 1,2-propanediol (de Bont *et al.*, 1982). More details on the regulation and properties of the epoxide hydrolase of strain AD1 will be reported elsewhere.

The actual substrates for the dehalogenating reactions were halohydrins. Halide liberation was found with eight different halohydrins when incubated with crude extracts of strain AD1 or AD2 grown on epichlorohydrin. The relevance of this activity was evident from its inducible character, the cellular levels of the enzyme being strongly elevated during growth with epichlorohydrin, as compared to citrate-grown cultures. Furthermore, a mutant of strain AD2 that had lost the ability to grow with epichlorohydrin no longer formed dehalogenase activity.

Theoretically, the product of the halohydrin dehalogenase reaction could be a diol or an epoxide, the first from a hydrolytic reaction and the second from an intramolecular substitution. Analysis of products formed in crude extracts of strain AD2 by GC-MS revealed that epoxides were produced from all five halohydrins tested. With strain AD1, epoxides were also found but not when chlorohydrins were added as substrates. The diols found as products in this case are considered to be due to further conversion by the epoxide hydrolase that is present in this organism. Dehalogenation of 3-chloro-1,2-propanediol to form glycidol has also been observed in rats (Jones, 1975). The latter compound may either undergo conjugation with liver glutathione to produce 2,3-dihydroxy-1-*S*-propylcysteine (Bray *et al.*, 1969; Jones & Fakhouri, 1979) or can be hydrolysed to glycerol (Jones, 1975).

Different routes for channelling epoxides in intermediary metabolism have been found (Weijers *et al.*, 1988). In incubations with extracts of strain AD1, but not of AD2, glycidol was converted, suggesting a role for the epoxide hydrolase, but no attempt was made to identify the product. Hydrolysis of glycidol to glycerol has been described (Bartnicki & Castro, 1969). Glycidol is hydrolysed at a lower rate than epichlorohydrin (Ross, 1950) probably making enzymic conversion necessary for growth.

The above mechanism for dehalogenation has also been observed with partially purified halohydrin epoxidase of a *Flavobacterium* sp. utilizing 2,3-dibromo-1-propanol (Castro & Bartnicki, 1968; Bartnicki & Castro, 1969). Both the enzyme described there and the dehalogenases identified in this paper have a very high activity for brominated vicinal alcohols. Since the relative dehalogenase activities of strain AD1, strain AD2 and the *Flavobacterium* sp. for chlorohydrins and bromohydrins are different, it is probable that the enzymes of the three organisms are not identical proteins.

Another striking difference between the activities identified here and the *Flavobacterium*

enzyme was the activity towards mono- and dichlorinated ketones of the halohydrin dehalogenases found in strains AD1 and AD2. It is unlikely that the organisms produce different dehalogenating enzymes for halohydrins and halo ketones since the relative activities of extracts for the chloroacetones and halohydrins were the same in citrate- and epichlorohydrin-grown cells. Moreover, the epichlorohydrin-negative mutant AD1M12 had lost its activity for chlorinated ketones, which makes the involvement of different dehalogenating enzymes in strain AD1 improbable. It remains to be determined whether the halohydrin dehalogenases catalyse conversion of halo ketones by using water as a nucleophile or use an enol tautomer as the actual substrate for an intramolecular substitution.

Further studies will involve kinetic aspects of epichlorohydrin and halohydrin degradation that are relevant for the application of epichlorohydrin-degrading organisms in treatment systems for the removal of these toxic compounds from waste streams.

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