Degradation of Halogenated Aliphatic Compounds by Xanthobacter autotrophicus GJ10

Janssen, Dick B.; Scheper, Alex; Dijkhuizen, Lubbert; Witholt, Bernard

Published in:
Applied and environmental microbiology

Publication date:
1985

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Mar. 1985, p. 673–677
0099-2240/85/030673-05$02.00/0

Vol. 49, No. 3

Degradation of Halogenated Aliphatic Compounds by *Xanthobacter autotrophicus* GJ10

DICK B. JANSSEN,[1]* ALEX SCHEPER,[1] LUBBERT DIJKHUIZEN,[2] AND BERNARD WITHOLT[1]

Department of Biochemistry, Groningen Biotechnology Center, University of Groningen, Nijenborgh 16, 9747 AG Groningen,[1] and Department of Microbiology, Groningen Biotechnology Center, University of Groningen, Kerklaan 30, 9751 NN Haren,[2] The Netherlands

Received 2 October 1984/Accepted 11 December 1984

A bacterium that is able to utilize a number of halogenated short-chain hydrocarbons and halogenated carboxylic acids as sole carbon source for growth was identified as a strain of *Xanthobacter autotrophicus*. The organism constitutively produces two different dehalogenases. One enzyme is specific for halogenated alkanes, whereas the other, which is more heat stable and has a higher pH optimum, is specific for halogenated carboxylic acids. Haloalkanes were hydrolyzed in cell extracts to produce alcohols and halide ions, and a route for the metabolism of 1,2-dichloroethane is proposed. Both dehalogenases show a broad substrate specificity, allowing the degradation of bromine- and chlorine-substituted organic compounds. The results show that *X. autotrophicus* may play a role in the degradation of organochlorine compounds and that hydrolytic dehalogenases may be involved in the microbial metabolism of short-chain halogenated hydrocarbons in microorganisms.

Chemical industries produce large amounts of short-chain halogenated aliphatic hydrocarbons, which are used as organic solvents, degreasing agents, pesticides, and intermediates for the synthesis of various other organic compounds. As is the case with so many industrial chemicals, haloalkanes have caused numerous cases of environmental pollution due to improper disposal of wastes, accidental spillage, or deliberate release. Contamination of soils, underground waters, and surface waters is frequently observed (12, 17).

Biodegradation is the main process by which xenobiotics disappear from the environment. However, information about the susceptibility of short-chain haloalkanes to biodegradation is scarce. Aerobic transformation has only been described for dichloromethane (6, 13, 20–22) and for 1,2-dichloroethane (11, 23). Anaerobic transformation of halogenated methanes in denitrifying cultures has been previously found (4). In methanogenic cultures, transformation was observed for halogenated methanes, 1,2-dichloroethane, 1,1,2,2-tetrachloroethane, and tetrachloroethylene (3, 5). In soil, chemical and biological conversions of haloalkanes that are used as biocides have been reported, and a reductive mechanism for the microbial removal of halide from 1,2-dibromoethane, 1,2-dibromo-3-chloropropane, and 2,3-dibromobutane was proposed (7).

Although aerobic degradation of haloalkanes seems possible and chloride release from 1,2-dichloroethane in crude cell extracts has been observed (11), the precise mechanism of the degradation of haloalkanes remains unclear (13, 19, 23). Dichloromethane degradation by a *Hyphomicrobium* strain involves nucleophilic substitution by a transferase that requires the presence of reduced glutathione for activity in crude extracts and produces *S*-chloromethyl glutathione. This compound was proposed to be converted nonenzymatically to formaldehyde and glutathione (22). Apart from chloride, the products of the dehalogenation of other haloalkanes have not been identified. The mechanism for haloalkane degradation could also be hydrolytic or oxidative (23); the latter is expected to be oxygen dependent and to produce an aldehyde, whereas hydrolytic dehalogenation would yield alcohols. Hydrolytic dehalogenation is known to occur during the metabolism of halogenated acetates and propionates (for a review, see reference 18) but has not been reported for haloalkanes (14).

We recently described the isolation of a bacterium that is able to grow aerobically with 1,2-dichloroethane as sole carbon and energy source (11). The organism constitutively produced enzymes that release chloride from 1,2-dichloroethane and chloroacetic acid, but the products and mechanism of these reactions are not known. The results described in this paper demonstrate that the organism belongs to the genus *Xanthobacter* and degrades haloalkanes to their corresponding alcohols by the action of a hydrolytic haloalkane dehalogenase with broad substrate specificity.

MATERIALS AND METHODS

Growth conditions. Cells of strain GJ10 (11) were grown aerobically at 30°C under rotary shaking. To prevent evaporation of substrates, cultivation was carried out in closed flasks, filled to one-fifth of their volume with medium. Growth medium contained per liter: 5.37 g of $Na_2HPO_4 \cdot 12H_2O$, 1.36 g of KH_2PO_4, 0.5 g of $(NH_4)_2SO_4$, 0.2 g of $MgSO_4 \cdot 7H_2O$, 5 ml of a salts solution, and 1 ml of a vitamin solution, as described previously (11). Carbon sources were added at 5 mM.

Halide levels in the culture fluids were determined by the colorimetric method of Bergmann and Sanik (2).

Preparation of crude extracts and enzyme assays. Cells were harvested by centrifugation (10 min at 10,000 × *g*), washed once with 10 mM Tris · SO_4 buffer (pH 7.5), and suspended in this buffer (1 ml/g of wet cells). After ultrasonic disruption of the cells, a crude extract was obtained by centrifugation (30 min at 45,000 × *g*).

Haloalkane dehalogenase activities were assayed at 30°C in 50 mM Tris sulfate buffer (pH 7.5) containing 5 mM substrate and enzyme in a final volume of 3 ml. At different time intervals, 0.5-ml samples were removed and assayed for halide levels.

* Corresponding author.

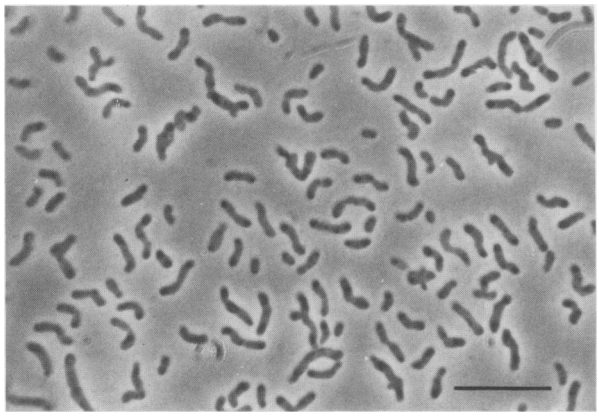

FIG. 1. Phase-contrast light micrograph of nutrient broth-grown cells of strain GJ10. Bar, 10 μm.

Halocarboxylic acid dehalogenase activities were assayed similarly but with 50 mM glycine · NaOH buffer (pH 9.0) instead of Tris buffer. All dehalogenase activities are expressed as micromoles of halide produced per minute per milligram of protein (milliunits per milligram).

Methanol and 2-chloroethanol dehydrogenase activities were assayed at 30°C by using the oxygen electrode. Phenazine methosulfate was used as an artificial electron acceptor, and the assay was carried out as described by Dunstan et al. (8). Dehydrogenase levels are expressed in micromoles of oxygen consumed per minute per milligram of protein (milliunits per milligram).

Protein concentrations were estimated by the method of Lowry et al. (15), with bovine serum albumin as the standard.

Gas chromatography. Degradation of halogenated alkanes in crude extracts was followed by gas chromatography after extraction of incubation mixtures with diethylether containing dodecane as an internal standard. Incubations were carried out as above in volumes up to 100 ml, using closed bottles. Samples of 2 ml were withdrawn and added to 2 ml of 1 mM dodecane in diethylether and 0.2 ml of 10% H_3PO_4. Etherial extracts were analyzed with a Varian 1400 gas chromatograph equipped with a flame ionization detector. A stainless steel column (4 m by 3 mm) packed with 10% Carbowax 20M on Chromosorb W was used at 110°C for determination of 1,2-dichloroethane and 2-chloroethanol. The same column was temperature programmed from 40 to 120°C at 10°C/min for analysis of 1-propanol and 1-butanol. A stainless steel column (2 m by 3 mm) packed with Chromosorb 102 was used at 150°C for separation of ethanol. In all cases, good separation of alcohols from their corresponding aldehydes, the solvent, and the substrate was obtained, as determined by the analysis of authentic standards.

Differential heat denaturation of dehalogenases. Heat treatment was carried out by incubation of crude extracts in a water bath. The thermostat control was manually adjusted from 30 to 70°C at 1°C/min. This gave rise to the rate of temperature increase indicated in Fig. 3. At different time intervals, samples were removed, chilled on ice, and assayed for residual dehalogenase activity.

Materials. Halogenated organic compounds were obtained from E. Merck AG, Darmstadt, Federal Republic of Germany, and from Janssen, Beersum, Belgium. The purity of haloalkanes was checked by gas chromatography and exceeded 99% for 1,2-dichloroethane, bromoethane, and 1-chlorobutane, and 1-chloropropane contained 7% of the 2-isomer. Non-halogenated chemicals were of analytical grade.

RESULTS

Characterization of strain GJ10. The initial characterization of the 1,2-dichloroethane-degrading organism (strain GJ10) described previously revealed that it is a gram-negative (somewhat resistant to decolorization), nonmotile bacterium that is able to grow on methanol, citrate, fructose, and sucrose but not on glucose, galactose, and lactose. Its yellow cellular pigmentation and pleomorphic appearance and the presence of refractile bodies (Fig. 1) suggest that the organism might belong to the genus *Xanthobacter*, which represents the nitrogen-fixing hydrogen bacteria (26). Strain GJ10 is capable of autotrophic growth with a mixture of H_2 and O_2 as energy source but not with NH_4^+-O_2 (Table 1). The organism can fix molecular nitrogen, both under autotrophic and heterotrophic growth conditions.

These results show that the organism should be classified as a strain of *Xanthobacter autotrophicus* (26). A comparison of the properties of strain GJ10 with the hypothetical median organism of 35 previously described strains of this species (26) shows a complete fit except for the oxidase reaction, which we scored negative (11) and was described positive for all tested strains (26) but was negative in a preceding report (25). Unlike *Xantobacter flavus* (16), strain GJ10 does not utilize glucose, histidine, or phenylalanine as a carbon source.

Apart from halogenated compounds, a number of organic chemicals could also support growth: methanol, ethanol, 1-propanol, 2-propanol, 1-butanol, ethylene glycol, acetone, and toluene. No growth was observed with 2-butanol, ethanolamine, pentane, hexane, 1-octanol, phenol, and benzene.

Degradation and utilization of halogenated compounds. It was determined whether strain GJ10 could utilize halogenated compounds that are structurally related to 1,2-dichloroethane and its possible degradation products. Since slight growth occurred on plain agar, growth tests were performed in liquid media supplemented with different halogenated compounds. The results (Table 2) show that besides 1,2-dichloroethane, a few other haloalkanes support growth: bromoethane, 1-chloropropane, 1,3-dichloropropane, and 1-chlorobutane. Three different halocarboxylic acids were

TABLE 1. Autotrophic growth and nitrogen fixation by strain GJ10

Growth medium[a]	Atmosphere	Growth[b]
MMV	70% H_2 + 10% CO_2 + 20% O_2	+
MMZV	75% N_2 + 10% H_2 + 10% CO_2 + 5% O_2	+
MMV + NH_4Cl	76% N_2 + 19% O_2 + 5% CO_2	−
MMZV + citrate	95% N_2 + 5% O_2	+
MMZV + methanol	95% N_2 + 5% O_2	+
MMZV + 2-chloroethanol	95% N_2 + 5% O_2	+
MMZV + 1,2-dichloroethane	95% N_2 + 5% O_2	+

[a] MMV is the minimal medium described in the text; MMZV is the same medium with $(NH_4)_2SO_4$ omitted. The compounds indicated were added at 5 mM.

[b] Growth was scored after 10 days of cultivation at 30°C. +, Increase of the optical density of the culture to at least 0.5 at 450 nm; −, no growth detectable.

TABLE 2. Utilization of halogenated compounds by strain GJ10

Growth medium[a]	Generation time[b,c] (h)	Halide production[b] (mM)	
		Inoculated	Sterile control
1,2-Dichloroethane	6.3	9.8	<0.1
2-Chloroethanol	5.3	5.0	0.1
Dichloroacetic acid	13	7.1	<0.1
Bromoethane	23	2.1	0.6
Dibromoacetic acid	6.5	8.8	<0.1
1-Chloropropane	5.6	4.2	<0.1
1,3-Dichloropropane	7.8	8.0	<0.1
2-Chloropropionic acid	9.0	3.5	0.2
1-Chlorobutane	6.9	5.0	<0.1

[a] Carbon sources were added at 5 mM; gases were added at 0.25 mmol/50 ml of medium.

[b] Growth and halide levels were scored after 10 days of cultivation in liquid medium at 30°C. No growth was observed with chloroacetic acid, trichloroacetic acid, methylchloride, ethylchloride, dichloromethane, 1,1-dichloroethane, 1,1,1-trichloroethane, 1,1,2-trichloroethane, 1,1,2,2-tetrachloroethane, 1,2-dibromoethane, 2-bromoethanol, bromoacetic acid, 2-chloropropane, 1,2-dichloropropane, and 3-chloropropene.

[c] Generation times were determined after inoculation of fresh medium with cells from a preculture grown on the same carbon source.

found to support growth. In all cases, growth was accompanied by halide release which strongly exceeded uninoculated controls.

The ability of strain GJ10 to utilize halogenated compounds was a very stable property in that 1,2-dichloroethane-negative variants were not detected after 24 serial transfers of the organism on nonselective medium.

Degradation of halogenated compounds in crude extracts. Since *X. autotrophicus* GJ10 utilized a number of different halogenated compounds for growth, the degradation of these compounds was tested in crude extracts. Halide production was observed with 1,2-dichloroethane (11), bromoethane, 1-chloropropane, 1,3-dichloropropane, 3-chloropropene, and 1-chlorobutane (Table 3). A number of chlorinated and brominated alkanoic acids were also dehalogenated in crude extracts, including bromoacetate and chloroacetate, which did not support growth.

TABLE 3. Dehalogenating activities of crude extracts prepared from 1,2-dichloroethane-grown cells toward various substrates and the effect of heat treatment

Substrate	Sp act (mU/mg of protein)		% Activity remaining after heat treatment[b]
	pH 7.5	pH 9.0	
1,2-Dichloroethane	310	210	0
Bromoethane	45	35	0
1-Chloropropane	68	57	0
1,3-Dichloropropane	167	110	0
Chlorobutane	91	54	0
3-Chloropropene	97	95	0
Chloroacetate	89	367	102
Dichloroacetate	167	471	102
Bromoacetate	94	520	100
Dibromoacetate	225	670	110
2-Chloropropionate	60	367	86

[a] Halide production at 30°C with different substrates (5 mM) was determined at pH 7.5 and 9.0.

[b] Crude extract of 1,2-dichloroethane-grown cells was heated up to 50°C as shown in Fig. 3. Remaining dehalogenase activities toward the substrates indicated (determined at pH 7.5) are given as percentages of the activity of untreated controls.

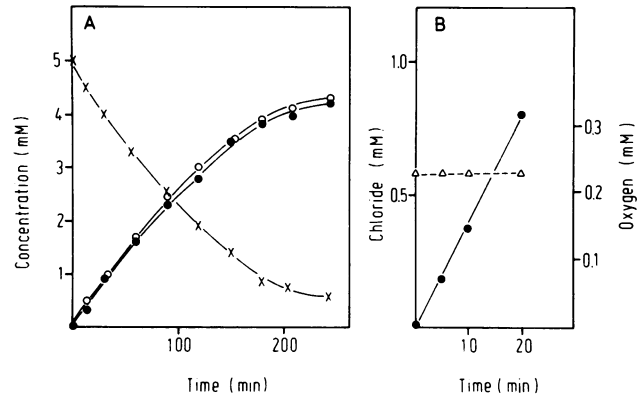

FIG. 2. Degradation of 1,2-dichloroethane in crude extracts of 1,2-dichloroethane-grown cells. (A) Production of 2-chloroethanol (○) and chloride (●) during degradation of 5 mM 1,2-dichloroethane (×). (B) Oxygen levels (△) during the production of chloride (●) from 1,2-dichloroethane in a biological oxygen monitor.

The products formed from bromoethane, 1-chloropropane, and 1-chlorobutane were analyzed by gas chromatography, and retention time comparison showed that these compounds were converted to ethanol, 1-propanol, and 1-butanol, respectively. Production of aldehydes was not observed. This suggests a hydrolytic mechanism for halide release.

Metabolism of 1,2-dichloroethane. To investigate the route of 1,2-dichloroethane metabolism, the compound was incubated with crude extract, and the products formed were analyzed by gas chromatography. It appeared that 1,2-dichloroethane was converted to 2-chloroethanol with no indication of chloroacetaldehyde formation. Chloride production from 1,2-dichloroethane was accompanied by the formation and disappearance of equimolar amounts of 2-chloroethanol and 1,2-dichloroethane, respectively (Fig. 2A). Furthermore, chloride production in crude extracts was not accompanied by oxygen consumption (Fig. 2B). Both results again indicate that dehalogenation is a hydrolytic reaction in this organism.

Further conversion of 2-chloroethanol is likely to proceed via chloroacetaldehyde and chloroacetate to glycolate. Crude extracts prepared from 1,2-dichloroethane-grown cells contained an ammonia-stimulated 2-chloroethanol dehydrogenase with optimal activity at pH 9.0. The enzyme was active with phenazine methosulfate as an artificial electron acceptor, whereas NAD and NADP were not active. The formation of this activity was induced together with methanol dehydrogenase during growth with 1,2-dichloroethane or methanol (Table 4) but not during growth with citrate.

TABLE 4. Methanol dehydrogenase and 2-chloroethanol dehydrogenase in *X. autotrophicus* GJ10

Carbon source	Sp act[a] (mU/mg of protein)	
	Chloroethanol dehydrogenase	Methanol dehydrogenase
1,2-Dichloroethane	518	535
Methanol	490	522
Citrate	<10	<10

[a] Specific activities of methanol dehydrogenase and 2-chloroethanol dehydrogenase were determined in crude extracts of cells grown on the carbon source indicated.

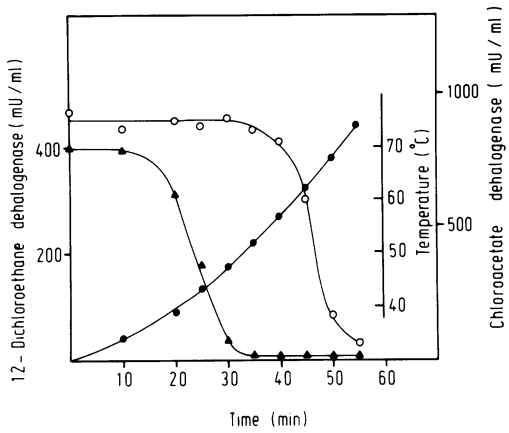

FIG. 3. Heat inactivation of dehalogenases. The levels of 1,2-dichloroethane dehalogenase (▲) and chloroacetate dehalogenase (○) were followed during heat treatment of crude extract prepared from 1,2-dichloroethane-grown cells. The temperature (●) was recorded.

Chloroacetaldehyde could possibly be converted to chloroacetate, followed by dehalogenation of this compound by the chloroacetate dehalogenase described previously (11; see below).

Identification of two dehalogenases. The dehalogenating activities toward various substrates were determined at two pH values, since 1,2-dichloroethane dehalogenase and chloroacetate dehalogenase show different pH optima (11). It appeared that the rate of halide liberation from halogenated carboxylic acids was three- to sixfold higher at pH 9 than at pH 7.5, and haloalkane-dehalogenating activities were lower at the higher pH for the substrates tested (Table 3). This suggests the existence of different dehalogenases for haloalkane and haloalkanoic acid degradation.

Heat denaturation studies confirmed the existence of at least two dehalogenases. The activity involved in chloride liberation from 1,2-dichloroethane was much more sensitive to heat treatment than chloroacetate dehalogenase, which was stable up to 50°C (Fig. 3). Treatment of crude extract up to 50°C also completely and selectively inactivated dehalogenase activity toward the other halogenated alkanes (Table 3).

Regulation of haloalkane dehalogenase formation. It was tested whether the formation of haloalkane dehalogenase activities was increased during growth with any of the respective substrates as carbon source. The results (Table 5) show that there is no significant induction of haloalkane dehalogenase synthesis by any of the compounds tested. The highest dehalogenase levels were observed in extracts prepared from autotrophically grown cells.

TABLE 5. Regulation of dehalogenase formation

Enzyme substrate	Specific dehalogenase activities[a] (mU/mg of protein)			
	Substrate[b]	1,2-DCE[c]	Citrate	CO_2[d]
1,2-Dichloroethane	232	232	226	386
Bromoethane	48	31	48	61
1-Chloropropane	76	63	111	180
1,3-Dichloropropane	256	190	244	317
1-Chlorobutane	84	78	77	10
Chloroacetic acid		416	462	1020

[a] Specific activities with the substrates indicated were determined with extracts from cells grown on the carbon sources indicated.
[b] Extract was prepared from cells grown on the substrate that was used for the enzyme assay.
[c] 1,2-DCE, 1,2-Dichloroethane.
[d] Autotrophic growth was with H_2-O_2 as the energy source (see Table 1 for details).

DISCUSSION

Our results demonstrate that the organism isolated on the basis of its ability to utilize 1,2-dichloroethane also utilizes a number of other haloalkanes for growth and is a nitrogen-fixing hydrogen bacterium that should be classified as a strain of *X. autotrophicus* (26). Carbohydrate utilization pattern, gram reaction, and colony and cell morphology (11; this paper) are in agreement with previously published data for *Xanthobacter* species (26). Up to now, the capability to degrade halogenated aliphatic compounds is mainly observed among members of the pseudomonads (10, 18) and has not been described for chemolithoautotrophic bacteria.

The degradation of haloalkanes in crude extracts was due to a haloalkane dehalogenase with broad substrate specificity. The enzyme is different from the haloalkanoate dehalogenase, as shown by differential heat inactivation. The haloalkane dehalogense catalyzes the hydrolytic release of halide from haloalkanes, resulting in the replacement of the halogen substituent by a hydroxyl group. This was shown for the degradation of 1,2-dichloroethane, 1-chlorobutane, 1-chloropropane, and bromoethane. In this respect, the enzyme is similar to the dehalogenases that are involved in the metabolism of halogenated carboxylic acids (9, 18). However, microbial dehalogenases that catalyze the hydrolytic conversion of haloalkanes to alcohols have not been described previously (14).

Further conversion of the compounds produced may proceed via oxidation to carboxylic acids. Ammonia-stimulated 2-chloroethanol dehydrogenase activity could be detected in 1,2-dichloroethane- and methanol-grown cells, suggesting the route for 1,2-dichloroethane degradation given in Fig. 4. Oxidation of 2-chloroethanol has also been found in 2-chloroethanol with pseudomonads (11, 24). The possibility

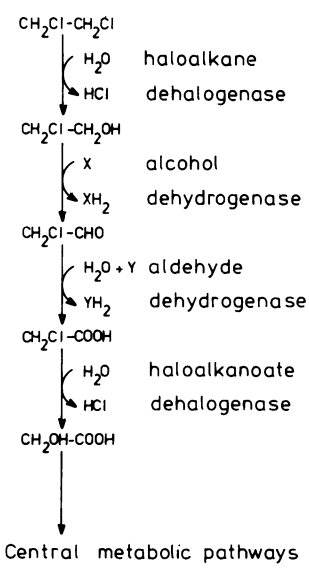

FIG. 4. Proposed route for the metabolism of 1,2-dichloroethane in *X. autotrophicus*.

that 2-chloroethanol is oxidized by a nonspecific methanol dehydrogenase is suggested by the observation that the two activities are simultaneously induced during growth with methanol or 1,2-dichloroethane, but this needs further study. The methanol dehydrogenase of a methylotrophic *Pseudomonas* species oxidizes 2-chloroethanol at 80% of the rate observed with methanol (1).

In a second *Xanthobacter* strain, isolated on the basis of its ability to utilize methanol, we could not detect dehalogenase activity (data not shown). This organism did not utilize halogenated compounds for growth. However, 2-chloroethanol dehydrogenase activity was present in extracts of methanol-grown cells of this strain. These results indicate that the production of dehalogenases is not a general characteristic of *Xanthobacter* species and that especially the receipt of the ability to produce these enzymes has enabled strain GJ10 to degrade haloorganics.

Both dehalogenases in strain GJ10 are synthesized constitutively. The dehalogenases were also present in autotrophically grown cells, and no induction by haloalkanes that served as carbon source was found. An intriguing question is how such enzymes involved in the degradation of xenobiotic haloalkanes have evolved. The haloalkane dehalogenase is clearly distinct from the haloalkanoate dehalogenase in the same organism. There is no overlap in substrate range, and the enzymes differ with respect to heat stability and do not copurify upon ammonium sulfate fractionation or ion-exchange chromatography (D. B. Janssen and S. Keuning, unpublished data). A further characterization of the haloalkane dehalogenase and a study toward the genetic basis of the ability to utilize haloalkanes should give more insight into this problem.

ACKNOWLEDGMENTS

We thank N. Panman for drawing the figures and K. Gilissen for photography.

This work was supported by the Program Commission for Biotechnology of The Netherlands.

LITERATURE CITED

1. **Anthony, C., and L. J. Zatman.** 1965. The microbial oxidation of methanol. The alcohol dehydrogenase of *Pseudomonas* sp. M27. Biochem. J. **96**:808–812.
2. **Bergmann, J. G., and J. Sanik.** 1957. Determination of trace amounts of chlorine in naphtha. Anal. Chem. **29**:241–243.
3. **Bouwer, E. J., and P. L. McCarty.** 1983. Transformation of 1- and 2-carbon halogenated aliphatic organic compounds under methanogenic conditions. Appl. Environ. Microbiol. **45**: 1286–1294.
4. **Bouwer, E. J., and P. L. McCarty.** 1983. Transformations of halogenated organic compounds under denitrification conditions. Appl. Environ. Microbiol. **45**:1295–1299.
5. **Bouwer, E. J., B. E. Rittmann, and P. L. McCarty.** 1981. Anaerobic degradation of halogenated 1- and 2-carbon organic compounds. Environ. Sci. Technol. **15**:596–599.
6. **Brunner, W., S. Staub, and T. Leisinger.** 1980. Bacterial degradation of dichloromethane. Appl. Environ. Microbiol. **40**:950–958.
7. **Castro, C. E., and N. O. Belser.** 1968. Biodehalogenation. Reductive dehalogenation of the biocides ethylene dibromide. 1,2-dibromo-3-chloropropane, and 2,3-dibromobutane in soil. Environ. Sci. Technol. **2**:779–783.
8. **Dunstan, P. M., C. Anthony, and W. T. Drabble.** 1972. Microbial metabolism of C1 and C2 compounds. The involvement of glycollate in the metabolism of ethanol and of acetate by *Pseudomonas* AM1. Biochem. J. **128**:99–106.
9. **Goldman, P., G. W. A. Milne, and D. B. Keister.** 1968. Carbon-halogen bond cleavage. III. Studies on bacterial halidohydrolases. J. Biol. Chem. **243**:428–434.
10. **Hardman, D. J., and J. H. Slater.** 1981. Dehalogenases in soil bacteria. J. Gen. Microbiol. **123**:117–128.
11. **Janssen, D. B., A. Scheper, and B. Witholt.** 1984. Biodegradation of 2-chloroethanol and 1,2-dichloroethane by pure bacterial cultures, p. 169–178. *In* E. H. Houwink and R. R. van der Meer (ed.), Innovations in biotechnology. Progress in industrial microbiology, vol. 20. Elsevier Biomedical Press, Amsterdam.
12. **Keith, L. H. (ed.).** 1976. Identification of organic pollutants in water. Ann Arbor Science, Ann Arbor, Mich.
13. **Klečka, G. M.** 1982. Fate and effects of methylene chloride in activated sludge. Appl. Environ. Microbiol. **44**:701–707.
14. **Leisinger, T.** 1983. Microorganisms and xenobiotic compounds. Experientia **39**:1183–1191.
15. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
16. **Malik, K. A., and D. Claus.** 1979. *Xanthobacter flavus*, a new species of nitrogen-fixing hydrogen bacteria. Int. J. Syst. Bacteriol. **29**:283–287.
17. **McConell, G., D. M. Ferguson, and C. R. Pearson.** 1975. Chlorinated hydrocarbons and the environment. Endeavour **34**:13–18.
18. **Motosugi, K., and K. Soda.** 1983. Microbial degradation of synthetic organochlorine compounds. Experientia **39**:1214–1220.
19. **Omori, T., and M. Alexander.** 1978. Bacterial dehalogenation of halogenated alkanes and fatty acids. Appl. Environ. Microbiol. **35**:867–871.
20. **Rittman, B. E., and P. L. McCarty.** 1980. Utilization of dichloromethane by suspended and fixed-film bacteria. Appl. Environ. Microbiol. **39**:1225–1226.
21. **Stucki, G., W. Brunner, D. Staub, and T. Leisinger.** 1981. Microbial degradation of chlorinated C1 and C2 hydrocarbons, p. 131–137. *In* T. Leisinger, A. M. Cook, R. Hütter, and J. Nüesch (ed.), Microbial degradation of xenobiotics and recalcitrant compounds. Academic Press, Inc., London.
22. **Stucki, G., R. Gälli, H. R. Ebersold, and T. Leisinger.** 1981. Dehalogenation of dichloromethane by cell extracts of *Hyphomicrobium* DM2. Arch. Microbiol. **130**:366–371.
23. **Stucki, G., U. Krebser, and T. Leisinger.** 1983. Bacterial growth on 1,2-dichloroethane. Experientia **39**:1271–1273.
24. **Stucki, G., and T. Leisinger.** 1983. Bacterial degradation of 2-chloroethanol proceeds via 2-chloroacetic acid. FEMS Microbiol. Lett. **16**:123–126.
25. **Tunail, N., and H. G. Schlegel.** 1974. A new coryneform hydrogen bacterium: *Corynebacterium autotrophicum* strain 7C. I. Characterization of the wild type strain. Arch. Microbiol. **100**:341–350.
26. **Wiegel, J., D. Wilke, J. Baumgarten, R. Opitz, and H. G. Schlegel.** 1978. Transfer of the nitrogen-fixing hydrogen bacterium *Cornyebacterium autotrophicum* Baumgarten et al. to *Xanthobacter* gen. nov. Int. J. Syst. Bacteriol. **28**:573–581.