APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Apr. 1992, p. 1220–1226
0099-2240/92/041220-07$02.00/0

Vol. 58, No. 4

Aerobic Vinyl Chloride Metabolism in *Mycobacterium aurum* L1

SYBE HARTMANS* AND JAN A. M. DE BONT

Division of Industrial Microbiology, Department of Food Science, Wageningen Agricultural University, P.O. Box 8129, 6700 EV Wageningen, The Netherlands

Received 24 October 1991/Accepted 28 January 1992

***Mycobacterium aurum* L1, capable of growth on vinyl chloride as a sole carbon and energy source, was previously isolated from soil contaminated with vinyl chloride (S. Hartmans et al., Biotechnol. Lett. 7:383–388, 1985). The initial step in vinyl chloride metabolism in strain L1 is catalyzed by alkene monooxygenase, transforming vinyl chloride into the reactive epoxide chlorooxirane. The enzyme responsible for chlorooxirane degradation appeared to be very unstable and thus hampered the characterization of the second step in vinyl chloride metabolism. Dichloroethenes are also oxidized by vinyl chloride-grown cells of strain L1, but they are not utilized as growth substrates. Three additional bacterial strains which utilize vinyl chloride as a sole carbon and energy source were isolated from environments with no known vinyl chloride contamination. The three new isolates were similar to strain L1 and were also identified as *Mycobacterium aurum*.**

Vinyl chloride is carcinogenic in experimental animals and humans (4, 25). Consequently, the U.S. Environmental Protection Agency has classified vinyl chloride as a priority pollutant.

The compound is produced on a very large scale by the chemicals industry, mainly for use in the production of the polymer polyvinyl chloride. Associated with these large-scale processes are inevitable losses to the environment. Vinyl chloride is a gas at ambient conditions (boiling point, 14°C at 1 atm [1 atm = 101.29 kPa]), and consequently a large percentage of the industrial losses are to the atmosphere. However, due to its relatively short half-life of 20 h in the troposphere, vinyl chloride does not accumulate in the atmosphere (14).

The presence of vinyl chloride in groundwater is attributed mainly to the biological reduction of polychlorinated ethenes (35). Complete dechlorination of tetrachloroethene (PCE) and trichloroethene (TCE) to ethene has been observed under methanogenic conditions, but the rate-limiting step is the conversion of vinyl chloride to ethene (12). More recently, reductive dechlorination of PCE to ethene via vinyl chloride in the absence of methanogenesis has also been reported (7). As a result of these reductive-dechlorination reactions, vinyl chloride concentrations of more than 1 mg/liter have been detected in groundwater contaminated with PCE and TCE (3, 24, 26). Therefore, further aerobic transformation of vinyl chloride could be of interest in the bioremediation of groundwater.

Aerobic vinyl chloride transformation has been observed with a wide range of microorganisms exhibiting monooxygenase activity. Methane- (10, 31), propane- (36), isoprene- (9), and ammonia- (34) utilizing bacteria have been shown to oxidize vinyl chloride, but no oxidation products were identified. By using purified soluble methane monooxygenase from *Methylosinus trichosporium* OB3b, the oxidation product of vinyl chloride was identified as chlorooxirane (11).

Aerobic mineralization of vinyl chloride by groundwater (5) and by a gram-positive, propane-grown bacterium (27) has been reported recently. To our knowledge, however, *Mycobacterium* strain L1 (17) is the only bacterial strain described so far which utilizes vinyl chloride aerobically as a sole source of carbon and energy. *Mycobacterium* strain L1 was isolated from soil that had been contaminated with vinyl chloride-containing water for a number of years.

The present report describes the isolation of three additional vinyl chloride-degrading mycobacteria. Furthermore, growth of *Mycobacterium* strain L1 on vinyl chloride and the initial step in vinyl chloride metabolism were studied by using chemostat cultures.

MATERIALS AND METHODS

Isolation of vinyl chloride-degrading strains. *Mycobacterium* strain L1 was previously isolated from soil that had been contaminated for several years with water containing vinyl chloride (5a, 17). Strain L1 has been deposited at the German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany, under accession number DSM 6695. The new strains were isolated by the following procedure. Soil (20 g) or water (20 ml) was mixed with 10 ml of mineral salts medium (MSM) which has been previously described (17) and put in serum bottles (about 130 ml) which were sealed with rubber septa. After the addition of 5 ml of vinyl chloride, the serum bottles were incubated statically in the dark at 30°C. After about 1 month, the contents were diluted fivefold with MSM. A 30-ml quantity of this diluted suspension was once again incubated under the same conditions. After a total of 2 months, 1 ml of these enrichment cultures was added to 30 ml of MSM in serum bottles of 130 ml. A 2-ml quantity of vinyl chloride was added, and cultures were incubated with gentle shaking at 30°C. The vinyl chloride concentration was determined weekly by analyzing headspace samples. Dilutions from cultures showing vinyl chloride degradation were plated on MSM agar plates which were incubated in a desiccator to which vinyl chloride (1% vol/vol) was added. After 2 and 4 weeks, the plates were inspected, and colonies which appeared to grow on vinyl chloride were streaked to purity by alternately plating them on glucose-yeast extract agar plates and on MSM agar plates which were incubated in a desiccator with vinyl chloride.

Maintenance and cultivation of strains. Vinyl chloride-degrading strains were grown on MSM agar plates at 30°C in a desiccator containing about 1% (vol/vol) vinyl chloride for about 1 month. Plates were sealed with paper adhesive tape.

* Corresponding author.

This usually prevented contamination of the plates with fungi during the prolonged incubation in the desiccator and also reduced the evaporation of water during subsequent storage outside the desiccator. Subculturing was routinely performed every 2 to 3 months. The MSM used in the enrichment cultures was the same as that described previously (17). In subsequent experiments, the following composition of MSM was used: per liter of deionized water, 3.88 g of K_2HPO_4, 2.13 g of $NaH_2PO_4 \cdot 2H_2O$, 2.0 g of $(NH_4)_2SO_4$, 0.1 g of $MgCl_2 \cdot 6H_2O$, 10 mg of EDTA, 2 mg of $ZnSO_4 \cdot 7H_2O$, 1 mg of $CaCl_2 \cdot 2H_2O$, 5 mg of $FeSO_4 \cdot 7H_2O$, 0.2 mg of $Na_2MoO_4 \cdot 2H_2O$, 0.2 mg of $CuSO_4 \cdot 5H_2O$, 0.4 mg of $CoCl_2 \cdot 6H_2O$, and 1 mg of $MnCl_2 \cdot 2H_2O$. In chemostat cultures, a buffer with a lower strength (containing 1.55 g of K_2HPO_4 and 0.85 g of $NaH_2PO_4 \cdot 2H_2O$ per liter) was used.

Growth experiments. Gaseous substrates were added at concentrations of 5% (vol/vol). With CO_2 as the carbon source, hydrogen gas (15% vol/vol) was also added. Liquid substrates were added at concentrations of 0.1% (vol/vol), and solid substrates were added at concentrations of 0.1% (wt/vol). Growth experiments with polychlorinated ethenes were performed by adding 50 μmol of substrate to Erlenmeyer flasks with a total volume of 300 ml and containing 100 ml of MSM and sealed with Teflon Mininert seals. Chemostat cultures were run at 30°C and pH 7 in a 3-liter Applikon fermentor with a working volume of 2 liters. The impeller speed was 750 rpm, and the dilution rate was 0.02 h^{-1} with 2% (vol/vol) vinyl chloride or ethene at an aeration rate of 200 ml/min. Growth rates were usually determined by monitoring substrate depletion curves (21). This method gave the same values as the growth rates that had been determined by monitoring the optical density at 660 nm and chloride liberation rates (21).

Oxidation experiments with whole cells. Degradation experiments with whole cells were performed at 30°C with vinyl chloride-grown cells of strain L1 freshly harvested from the chemostat and washed with 50 mM phosphate buffer (pH 7.0). The oxidation of polychlorinated ethenes (5 μl) was performed in 300-ml flasks fitted with Teflon Mininert valves containing cells in 25 or 50 ml of 50 mM potassium phosphate buffer (pH 7.0). The oxidation of vinyl chloride by other alkene-utilizing bacteria was performed with cells which had been stored at −20°C and cultivated as described previously (15, 33).

Preparation of cell extracts. Cells were harvested from the chemostat or from batch cultures in the exponential growth phase by centrifugation at 16,000 × *g* for 10 min at 4°C, resuspended in about 200 ml of 50 mM potassium phosphate buffer (pH 7.0), and centrifuged once again. The pellet was subsequently resuspended in approximately 6 ml of the same buffer containing 2 mM dithiothreitol. The concentrated washed-cell suspension was placed on ice and disrupted by ultrasonication with a Branson B-12 Sonifier with a power input of 10 W eight times for 15 s each time. Whole cells and cell debris were removed by centrifugation at 27,000 × *g* for 20 min at 4°C. The supernatant was used directly as the cell extract in the enzyme activity assays.

Enzyme assays. All assays were performed at 30°C. Spectrophotometric assays were performed on a Perkin-Elmer 550A spectrophotometer. Activities are expressed in nanomoles of product formed or of substrate consumed per minute per milligram of protein.

(i) **Alkene monooxygenase.** Alkene monooxygenase was assayed by analyzing epoxypropane formation. Assays were done in 35-ml serum bottles sealed with rubber septa. The reaction mixture contained 2 μmol of NADH, cell extract, and 50 mM potassium phosphate buffer (pH 7.3) in a total volume of 1 ml. The serum bottles were incubated at 30°C in a shaking water bath. After 2 min in the water bath, the reaction was started by the addition of 1 ml of propene via the rubber septum. Epoxypropane formation was determined by analyzing headspace samples of 200 μl every 2 to 3 min during a total incubation time of about 20 min. The epoxypropane formation rate was constant during this time period.

(ii) **Epoxyethane dehydrogenase.** Epoxyethane dehydrogenase was assayed by determining epoxyethane consumption. The assay described by de Bont and Harder (6) was slightly modified. The following substances were added to a serum bottle with a total volume of 35 ml: 450 μl of 100 mM Tris-HCl (pH 8.5), 50 μl of 10 mM NAD^+, 50 μl of 10 mM coenzyme A, 50 μl of 2 mM flavin adenine dinucleotide, and water to give a total volume of 1 ml after the addition of extract. The bottle was sealed with a rubber septum and flushed with nitrogen for 2 to 3 min to remove most of the oxygen, and 1 ml of 1% (vol/vol) epoxyethane in nitrogen gas was added. The bottles were subsequently incubated in a shaking water bath, and after 2 min the reaction was started by the addition of cell extract (0.2 to 0.4 ml) through the septum with a syringe. The epoxyethane consumption rate was determined by analyzing headspace samples either until all of the epoxyethane was consumed or for a maximum of 50 min. The epoxyethane consumption rate was constant during this time period.

(iii) **Isocitrate lyase and isocitrate dehydrogenase.** Isocitrate lyase and isocitrate dehydrogenase were assayed as described previously (19).

Identification of chlorooxirane. The following experiment was performed, starting with a steady-state culture of *Mycobacterium aurum* L1 operated at a dilution rate of 0.016 h^{-1} and an airflow of 85 ml/min containing vinyl chloride (7,500 ppm). The dilution rate was reduced to zero, and 0.5 liters of MSM was added to decrease the volume of the gas phase and thereby decrease the residence time of the air passing through the fermentor. At the same time, the inlet vinyl chloride concentration was increased to 11,000 ppm. After 8 h at these conditions, the vinyl chloride conversion was 97.4%. The airflow into the fermentor was subsequently increased to 200 ml per min with 6,750 parts of vinyl chloride per million, resulting in a conversion of 68%. The air leaving the fermentor was passed through 5 ml of ethanediol containing 4-(4-nitrobenzyl)pyridine for 30 min to trap any chlorooxirane formed. The air-vinyl chloride flow was subsequently replaced by 100 ml of pure air per min for 43 min. After these 43 min, the original air-vinyl chloride mixture (200 ml/min; 6,750 ppm) was passed through the fermentor again, and the gas phase leaving the fermentor was passed through 5 ml of ethanediol containing 4-(4-nitrobenzyl)pyridine for 30 min. The vinyl chloride conversion gradually decreased to 31% over 30 min. The ethanediol solutions were immediately analyzed for the chlorooxirane adduct of 4-(4-nitrobenzyl)pyridine as described by Barbin et al. (1).

Analytical methods. Biomass dry weights were routinely determined by measuring the absorbance of an appropriate dilution in MSM at 660 nm with a Perkin-Elmer 550A spectrophotometer. Dry weight versus optical density was assumed to be linear below an absorbance of 0.5, which corresponded with 130 mg (dry weight) per liter (determined with glucose-grown cells after 24 h at 105°C).

Concentrations of vinyl chloride, ethene, epoxyalkanes, and chlorinated ethenes were determined by analyzing 100-μl headspace samples on a Packard 430 gas chromato-

graph fitted with a stainless-steel Porapak R column (100/120 mesh; 110 cm by 1/8 inch inner diameter) and a flame ionization detector. The oven temperature was 180°C (200°C for the analysis of polychlorinated ethenes), and the carrier gas was N_2, at a flow rate of 20 ml/min. Total glutathione was quantified as described by Tietze (30). Protein was quantified by the Bradford (2) method with bovine serum albumin as the standard.

Chemicals. Vinyl chloride (chloroethene) with a purity of 99.95%, propene, ethene, and epoxyethane were from Hoek Loos, Schiedam, The Netherlands. Vinyl bromide and 1,1-, *cis*-, and *trans*-1,2-dichloroethene were from Janssen Chimica, Beerse, Belgium. 4-(4-Nitrobenzyl)pyridine, DL-isocitrate, and coenzyme A were from Sigma, St. Louis, Mo. Dithiothreitol, NAD^+, NADH, $NADP^+$, and ATP were from Boehringer, Mannheim, Germany. Other chemicals were from Merck, Darmstadt, Germany.

RESULTS

Isolation and characterization of vinyl chloride-degrading strains. In the present investigation, a series of inocula from different sources without any known history of vinyl chloride contamination were used in enrichment cultures with vinyl chloride as the carbon source. Three vinyl chloride-degrading strains designated VC2, VC3, and VC4 were isolated from the 20 different inocula used. They were isolated from soil which had been treated with 1,3-dichloropropene for 20 years (VC2), from the sludge of an aerobic wastewater treatment plant mainly treating domestic wastewater (VC3), and from the River Rhine sampled at Wageningen (VC4), respectively. The new isolates all formed yellow colonies on agar plates, as did the previously isolated *Mycobacterium* strain L1. All four strains were tentatively identified as *M. aurum* at the National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands. The colors and colony morphologies of the new isolates differed somewhat, indicating that they were different strains.

None of the ethene- or propene-utilizing strains of the genera *Mycobacterium* and *Xanthobacter* previously isolated in our laboratory (15, 32) could grow with vinyl chloride as the sole carbon source. Alkene-grown cells did, however, oxidize vinyl chloride at initial rates similar to those of vinyl chloride-grown cells of strain L1. Ethene-grown cells of *Mycobacterium* strain E3 and propene-grown cells of *Xanthobacter* strain Py2 oxidized vinyl chloride with initial specific activities of 37 and 33 nmol min^{-1} mg of $protein^{-1}$, respectively.

The four vinyl chloride-utilizing strains exhibited the same pattern of growth substrate utilization. Ethene, ethanol, acetate, 1-propanol, propionate, pyruvate, and glycerol were all utilized as sole sources of carbon and energy. All strains grew autotrophically on H_2. Methane, methanol, ethane, ethanediol, glycolate, glycine, propene, 2-propanol, 1,2-propanediol, chloroethane, chloroethanol, and chloroacetate (0.05%, wt/vol) did not support growth.

Vinyl chloride degradation kinetics. As no obvious differences among the four strains were observed, subsequent experiments were performed with strain L1. Growth rates with vinyl chloride were determined a number of times, and they varied between 0.03 and 0.06 h^{-1}. A growth rate of about 0.04 h^{-1} was determined most frequently. The observed growth rates were influenced by the history of the inoculum. Prolonged subculturing in liquid batch cultures with vinyl chloride as the carbon source resulted in an increase of the growth rate with vinyl chloride, whereas subculturing on glucose-yeast extract agar plates resulted in a decreased growth rate with vinyl chloride. Both effects were reversible.

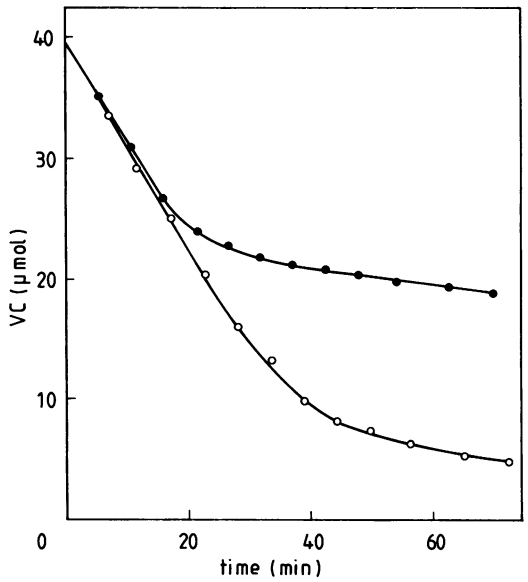

FIG. 1. Vinyl chloride degradation by washed cells of *M. aurum* L1. ●, total volume of liquid phase (3 ml) containing 16 mg of freshly harvested vinyl chloride-grown cells in phosphate buffer; ○, same composition plus 16 mg of heat-inactivated cells.

Strain L1 was grown in a vinyl chloride-limited chemostat at a number of dilution rates between 0.012 and 0.032 h^{-1} for several months. After an apparent steady state was obtained, the vinyl chloride concentration in the air from the chemostat was determined several times during a time period which varied from 1 to 5 days. From these data and from the maximum specific growth rate of 0.040 h^{-1}, an apparent K_s of 3.2 μM was calculated. In calculating the K_s, the gas-phase vinyl chloride concentration in the chemostat was assumed to be in equilibrium with the liquid-phase concentration.

Vinyl chloride metabolism. Freshly harvested, vinyl chloride-grown, washed cells of strain L1 oxidized vinyl chloride at a rate of 55 nmol min^{-1} mg of dry $weight^{-1}$. Very rapid inactivation of vinyl chloride degradation was, however, observed (Fig. 1). Incubation of cells at the same densities for 1 h at 30°C before vinyl chloride was added gave an almost identical vinyl chloride degradation curve (results not shown). Inactivation could be delayed by adding boiled cells (Fig. 1). No vinyl chloride degradation was observed when only boiled cells were present (results not shown). We concluded from this experiment that the inactivation was caused by a toxic metabolite of vinyl chloride metabolism which could accumulate extracellularly.

The most obvious toxic product that could be formed from vinyl chloride is chlorooxirane, the product of vinyl chloride epoxidation, or its rearrangement product, chloroacetaldehyde. The involvement of chlorooxirane in vinyl chloride metabolism would require the presence of monooxygenase activity in vinyl chloride-grown cells. It was previously shown that vinyl chloride- and ethene-grown cells of strain L1 oxidized propene to 1,2-epoxypropane (37), indicating that alkene monooxygenase was present in cells grown on both substrates. Alkene monooxygenase could also be as-

TABLE 1. Enzyme activities in cell extracts of *M. aurum* L1 grown on various carbon sources

Enzyme	Activity (nmol/min/mg of protein) in extracts from cells grown on:			
	Vinyl chloride	Ethene	Acetate	Succinate
Alkene monooxygenase	2.35 (7.8)[a] 2.02 (5.2) 1.13 (2.6)	1.60 (12.2) 1.32 (8.1) 0.96 (4.1)	ND	ND
Epoxyethane dehydrogenase	2.22 (5.2) 0.99 (2.6)	— (8.1) 7.4 (4.1)	ND	ND
Isocitrate lyase	111	89	87	3
Isocitrate dehydrogenase	245	272	550	346

[a] Values in parentheses indicate the amount of protein (mg) in the assay. Vinyl chloride- and ethene-grown cells were from chemostat cultures (D = 0.02 h^{-1}); acetate and succinate cells were batch grown. ND, not detectable; —, reaction too fast to allow reliable determination of activity.

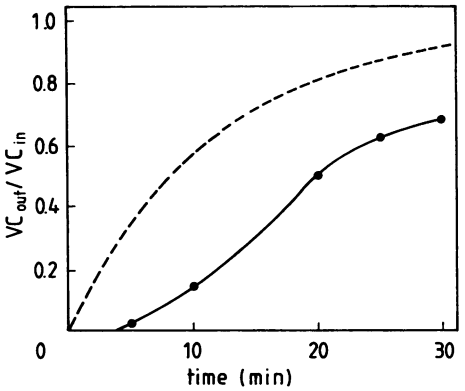

FIG. 2. Vinyl chloride outlet concentrations upon restoring the vinyl chloride supply to a culture of *M. aurum* L1 which had been without vinyl chloride for 43 min (——) and the theoretical outlet concentrations that would be observed if no vinyl chloride were transformed after the vinyl chloride supply was restored (– – –).

sayed in crude extracts of ethene- or vinyl chloride-grown cells (Table 1). As alkene monooxygenase is a multicomponent enzyme (22), the observed specific activities vary with the protein content of the assay (Table 1). Vinyl chloride oxidation by dialyzed crude extracts was dependent on the presence of both oxygen and NADH. No monooxygenase activity was detected in cells grown on acetate or succinate.

On several occasions, after an interruption in the vinyl chloride supply to the continuous culture, a significant drop in vinyl chloride conversion was observed. This could be the result of monooxygenase inactivation due to a temporary accumulation of chlorooxirane upon restoration of the vinyl chloride supply to the culture.

To verify the presumed formation of chlorooxirane, the air leaving the fermentor was analyzed by passing it through a solution containing 4-(4-nitrobenzyl)pyridine in ethanediol as described by Barbin et al. (1). This was done with air from the fermentor prior to the vinyl chloride supply being interrupted for 43 min and also with the air from the fermentor directly after the vinyl chloride supply was restored. The broken line in Fig. 2 indicates the theoretical outlet concentrations that would be observed if no vinyl chloride were transformed after the vinyl chloride supply was restored. The characteristic spectrum of the chlorooxirane adduct (1) was detected only in the solution through which the air from the fermentor had been passed after the interruption in the vinyl chloride supply (Fig. 3). This confirms that chlorooxirane is the oxidation product of vinyl chloride. It also indicates that the enzyme responsible for the degradation of the chlorooxirane is unstable or that the activity of this enzyme is very sensitive to changes in the intracellular environment (e.g., cofactor levels) due to the absence of an exogenous carbon and energy source.

Mammalian metabolism of chlorooxirane involves the formation of *S*-formyl methyl glutathione (8), either directly or via chloroacetaldehyde. Extracts of batch-grown cultures of strain L1, however, contained only about 1 μmol of total glutathione per gram of protein after growth on vinyl chloride, ethene, or acetate.

The growth yield of *M. aurum* L1 with ethene and vinyl chloride as substrate was determined by using closed batch cultures. The observed yields were 0.77 and 0.22 g of biomass formed per g of substrate utilized, respectively. Calculation of the growth yields on a molar basis results in 21.7 and 13.8 grams of biomass formed per mole of ethene or vinyl chloride utilized, indicating that the oxidation of vinyl chloride yields less ATP than the oxidation of ethene.

Oxidation of polychlorinated ethenes by *M. aurum* L1. Table 2 shows the initial oxidation rates of various chlorinated ethenes by vinyl chloride-grown cells of *M. aurum* L1. Ethene oxidation resulted in epoxyethane accumulation, and with *trans*-1,2-dichloroethene as the substrate, an unidentified compound, probably the epoxide (23), accumulated. Vinyl bromide and the compounds listed in Table 2 were also tested as growth substrates with strain L1. Growth was determined as substrate depletion. With ethene, vinyl chloride, and vinyl bromide, more than 50% of the substrate was consumed within 6 days. With the polychlorinated ethenes as growth substrates, no significant substrate degradation or carbon dioxide formation could be observed, even after 3 weeks.

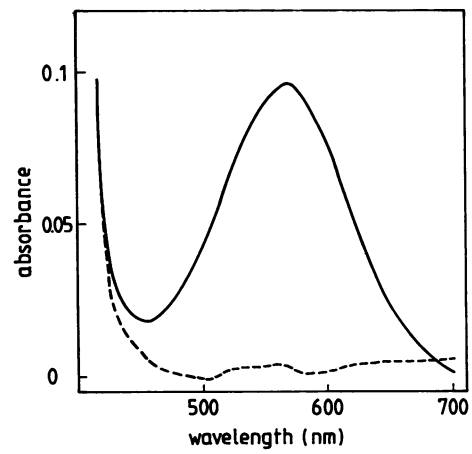

FIG. 3. Absorption spectra of 4-(4-nitrobenzyl)pyridine adducts in ethanediol solutions through which the air from the fermentor was passed prior to the interruption in the vinyl chloride supply (– – –) and directly after restoration of the vinyl chloride supply (——).

TABLE 2. Whole-cell oxidation rates of chlorinated alkenes by vinyl chloride-grown *M. aurum* L1 cells

Substrate	Initial oxidation rate (nmol/min/mg of dry weight)
Vinyl chloride (chloroethene) (350)	55
1,1-Dichloroethene (165)	10
cis-1,2-Dichloroethene (860)	30
trans-1,2-Dichloroethene (425)	25
Trichloroethene (320)	<1

[a] Values in parentheses indicate the initial substrate concentration (μM), calculated by using the partition coefficients determined by Gossett (13).

DISCUSSION

Isolation of vinyl chloride utilizers. In contrast to the previously isolated strain L1, the three new strains were isolated from environments not known to be contaminated with vinyl chloride. Prolonged contamination with vinyl chloride is therefore apparently not a prerequisite for the evolution of the vinyl chloride degradative pathway in bacteria.

It is remarkable that all four isolates were strains of *M. aurum*, although colony morphologies and pigmentations indicated that the four strains were not identical. A similar situation has, however, been observed when ethene is used as the carbon source in enrichment cultures. Until now, all strains isolated from enrichment cultures with ethene as the sole carbon and energy source were identified as mycobacteria (18). A *Xanthobacter* sp. isolated from an enrichment culture with propene as the sole carbon and energy source was also capable of growth with ethene (32), but the growth rate was much lower than the growth rates of the ethene-utilizing mycobacteria. None of the ethene- or propene-utilizing bacteria tested could grow with vinyl chloride.

Until now, there were no reports concerning the isolation of pure cultures of vinyl chloride-utilizing microorganisms in the literature. This may be due in part to the low concentrations of vinyl chloride used in the published studies concerning aerobic degradation of vinyl chloride. Davis and Carpenter (5), for example, used 0.1 and 1 μg of vinyl chloride per liter. This concentration is very low compared with the apparent K_s of strain L1 for vinyl chloride of 200 μg/liter. We have observed the induction of vinyl chloride degradation by glucose-grown cells of strain L1 at a concentration of 10 μg/liter but have not tested lower concentrations. Phelps et al. (27) used 1 mg of vinyl chloride per liter in the presence of propane (5% vol/vol), but their isolate was apparently not capable of growth on vinyl chloride as the sole source of carbon and energy. The recovery of labeled CO_2 (5, 27), assuming that vinyl chloride is also epoxidated in these cases, could indicate that these cultures had enzymes which very efficiently transform chlorooxirane, but it more likely indicates that these cultures were able to metabolize the products formed from the alkylation of glutathione (28) or coenzyme A (29) by chlorooxirane or chloroacetaldehyde, the rearrangement product of chlorooxirane.

The observed variation in the growth rate of strain L1 with vinyl chloride, which depended on the maintenance conditions of the culture, probably indicates that the regulation of vinyl chloride metabolism in *M. aurum* L1 is still genetically unstable. Indeed, we have observed that subculturing strain L1 on agar slants with yeast extract-glucose for more than 2 years resulted in the loss of the capacity of the total population to grow on vinyl chloride.

Vinyl chloride metabolism. Alkene monooxygenase activity was present in crude extracts of vinyl chloride-grown *M. aurum* L1. Combined with the observation that vinyl chloride degradation can be competitively inhibited by the addition of ethene or propene, which are both oxidized to the corresponding epoxides, this is strong evidence that the initial step in vinyl chloride metabolism is indeed catalyzed by alkene monooxygenase. The presumed product of vinyl chloride oxidation, chlorooxirane, is a very reactive and unstable compound (rearranging to chloroacetaldehyde with a half-life of 1.6 min in Tris-HCl buffer (pH 7.4) at 37°C [1]), indicating that, during growth with vinyl chloride, the epoxide must be metabolized very effectively to prevent the accumulation of toxic levels within the cell. However, on the basis of the inactivation of washed cells degrading vinyl chloride (Fig. 1), this very effective enzyme would also appear to be very unstable, rapidly losing its activity in the absence of an inducer. This hypothesis was confirmed by the experiment in which the vinyl chloride supply to a chemostat culture was interrupted for only 43 min. After the vinyl chloride supply was restored, chlorooxirane was detected in the air from the chemostat. No chlorooxirane could be detected prior to the interruption (Fig. 3). Apparently, this time period was already long enough to allow (some) loss of activity of the chlorooxirane transforming enzyme, consequently resulting in the accumulation of the inhibitory epoxide. In studying the inhibitory effects of the less reactive epoxide 1,2-epoxypropane, it was previously demonstrated that the inhibitory effect on the monooxygenases examined was much stronger than that on other physiological functions of the cell (16). Although not examined in the present investigation, this is probably also the case with chlorooxirane.

The apparent instability of the chlorooxirane-degrading enzyme, in combination with the reactivity and instability of the epoxide itself, makes further elucidation of the vinyl chloride degradative pathway very difficult. The observed presence of epoxyethane dehydrogenase activity in extracts of vinyl chloride-grown cells does not necessarily indicate the involvement of this enzyme in vinyl chloride metabolism. In the ethene-utilizing *Mycobacterium* strain E3, the monooxygenase and the epoxide dehydrogenase are both induced by epoxyalkanes (unpublished results). A number of other possible enzymatic transformations of epoxides can, however, be ruled out. Hydrolysis or isomerization (20) of the epoxide would result in glycolaldehyde and chloroacetaldehyde, respectively. As strain L1 does not grow on ethanediol, glycolate, or chloroethanol, this would seem unlikely to occur. Only very low levels of glutathione could be detected in strain L1 grown on various substrates, indicating that glutathione-dependent transformation of chlorooxirane is also unlikely.

Oxidation of other chlorinated ethenes. Washed cells of vinyl chloride-grown *M. aurum* L1 oxidized dichloroethenes at rates which were in the same range as vinyl chloride oxidation (Table 2). TCE was not oxidized at a detectable rate at the concentration tested. However, an isoprene-utilizing strain, which also exhibits alkene monooxygenase activity, was recently shown to oxidize chlorinated ethenes, including TCE (9). With the exception of 1,1-dichloroethene, which was oxidized at the same rate, the oxidation rates of vinyl chloride (25%) and the 1,2-dichloroethenes (2 to 10%) were somewhat lower than the rates we observed with strain L1 (Table 2). The TCE oxidation rate was rather low, 0.15 nmol min^{-1} mg $protein^{-1}$ when the TCE concentration was 6 μM (9).

Conclusions. Vinyl chloride metabolism in *M. aurum* L1

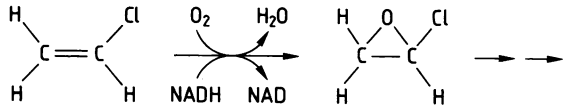

FIG. 4. Initial step in vinyl chloride metabolism of *M. aurum* L1, catalyzed by alkene monooxygenase.

proceeds via oxidation of vinyl chloride to chlorooxirane by alkene monooxygenase (Fig. 4). The apparent instability of the enzyme responsible for the further metabolism of chlorooxirane in combination with the toxic characteristics of this compound can result in inhibition of the monooxygenase-oxidizing vinyl chloride. This aspect of vinyl chloride degradation by *M. aurum* L1 does not favor the application of this strain in the removal of vinyl chloride from waste gases, as the concentration can fluctuate to a great extent, but it need not be problematic in the bioremediation of groundwater. Furthermore, strain L1, and alkene-utilizing strains in general, may prove to be of interest in view of the cometabolic degradation of various other chlorinated ethenes.

ACKNOWLEDGMENTS

We thank D. G. Groothuis (RIVM, Zeist, The Netherlands) for identifying the vinyl chloride utilizers, G. Lebbink (IB, Haren, The Netherlands) for supplying the 1,3-dichloropropene-treated soil, and Paul van Eijck and Ronald Niermans for performing some of the experimental work.

The investigations were financed in part by the Dutch Ministry of Housing, Physical Planning and Environment.

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