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NOTES

Aerobic Mineralization of Vinyl Chloride by a Bacterium of the Order *Actinomycetales*

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A gram-positive branched bacterium isolated from a trichloroethylene-degrading consortium mineralized vinyl chloride in growing cultures and cell suspensions. Greater than 67% of the [1,2-^{14}C]vinyl chloride was mineralized to carbon dioxide, with approximately 10% of the radioactivity appearing in cell biomass and another 10% appearing in ^{14}C-aqueous-phase products.

The U.S. Environmental Protection Agency has listed vinyl chloride (VC) as a priority pollutant. VC is a common groundwater contaminant associated with reductive dechlorination of chlorinated ethenes (1, 14). The fate of VC is poorly understood; its volatility and low solubility in the aqueous phase has led researchers to speculate that it has a poor biotransformation potential (3), with the rate and extent of degradation appearing low under anaerobic conditions (2, 7, 14). Aerobic biodegradation of VC by *Mycobacterium* sp. strain L1 was reported by Hartmans et al. (8). Recently, several investigators reported the disappearance of VC from mixed cultures (6, 11), bioreactors (10), and pure cultures (12, 13, 15) without demonstration of transformation products. Recently, VC mineralization was reported in samples of groundwater (4). We report that strain Sm-1, a member of the order *Actinomycetales*, mineralized VC to carbon dioxide in growth and cell suspension experiments. Strain Sm-1 also incorporated 10% of the [^{14}C]VC pool into biomass.

Chemicals were of reagent grade and were obtained from Mallinckrodt Inc. (Paris, Ky.) or Difco (Detroit, Mich.). VC dissolved in methanol was obtained from Supelco (Bellefonte, Pa.), and VC in N_2 gas (10% [vol/vol]) was from Matheson (East Rutherford, N.J.). [1,2-^{14}C]VC dissolved in toluene (0.53 mCi/mmol) was obtained from New England Nuclear (Boston, Mass.).

Total and radioactive carbon dioxide were determined by gas chromatography-gas proportional counting by using a Shimadzu GC 8A gas chromatograph and a Packard 894 gas proportional counter (5, 9). VC was quantified by using a Shimadzu GC 9A gas chromatograph equipped with a photoionization detector (HNU Systems, Newton, Mass.). The gas chromatograph was equipped with a 2.5-m-long, 3.2-mm-diameter Poropak T column. Verification of $^{14}CO_2$ and [^{14}C]VC was accomplished by switching the photoionization detector from nitrogen to helium carrier gas and connecting it to the gas proportional counter. Propane was analyzed by using a Shimadzu GC 9A gas chromatograph equipped with a flame ionization detector (5). Data were analyzed by using a Nelson Analytical 2600 chromatograph system (Perkin Elmer). All experiments were performed by using serum vials sealed with Teflon-lined septa (Alltech, Deerfield, Ill.).

Strain Sm-1 was obtained from a trichloroethylene-degrading consortium enriched from contaminated subsurface sediments (5). The consortium repeatedly degraded VC in batch and bioreactor experiments. Strain Sm-1 was isolated by using a basal medium (5) with propane as a substrate. Sm-1 is a gram-positive asporogenous rod which exhibits branching during growth. Stationary-phase cells exhibit a rod-to-coccus morphogenesis. The organism is obligately aerobic, nonmotile, and catalase positive. Cellular fatty acids consisted of normal 14-to-22-carbon-long saturated and unsaturated fatty acids plus the branched-chain 10-methyl octodecanoic acid, indicating possible affiliation with the genus *Rhodococcus*. The bacterium grows on complex media or propane but not on methane, is mesophilic, and grows in a pH range of 5.5 to 10.

Strain Sm-1 degraded VC during successive growth experiments by utilizing propane (5% [vol/vol] headspace) as an energy source to which 1 mg of VC liter^{-1} was added, and

TABLE 1. Degradation of VC by cell suspensions of isolate Sm-1[a]

Growth substrate	Peak area	% VC loss[b]	Growth substrate	Peak area	% VC loss[b]
Propane (3%)			Glucose (0.1%)		
Controls	33,600		Controls	38,100	
Sm-1	7,600	78	Sm-1	460	99
Propane (3%)			Acetate (0.4%)		
Controls	64,000		Controls	37,000	
Sm-1	7,700	88	Sm-1	18,000	53
Propane (3%)			Yeast extract + Trypticase (0.2%)		
Controls	51,000		Controls	34,000	
Sm-1	20,000	61	Sm-1	33,000	3

[a] Cultures were grown for 1 week, centrifuged, and suspended in 5.0 ml of phosphate-buffered basal salts at 0.8 to 1.0 g (dry weight) per liter. Suspensions were incubated for 1 week in 25-ml serum vials which contained 1 mg of VC per liter.

[b] Values represent percent differences between averages of triplicate samples and averages of an uninoculated vial and an inoculated control inhibited with 0.1% Formalin and sodium azide.

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TABLE 2. Mineralization of radiolabeled VC by isolate Sm-1[a]

Sample	Total CO_2 (% headspace)	% VC loss	Total dpm/vial (10^3) of:			% ^{14}C-products recovered
			$^{14}CO_2$	^{14}C-soluble end products	^{14}C-biomass	
Growth expts						
Controls						
Uninoculated	1.5	<10	<4	1.1	0.0	0.07
Inhibited	1.6	<10	<4	2.3	0.6	0.19
Test culture	5.0	99.9	989	NA[b]	NA	>66
Test culture	5.3	>99.9	1,180	93	170	96
Test culture	5.8	99.9	974	91	143	81
Test culture	6.1	>99.9	1,100	76	96	85
Cell suspensions						
Control						
Uninoculated	1.5	<10	<4	0.9	0.0	0.06
Inhibited	1.8	<10	<4	2.1	0.6	0.18
Test suspension	3.9	92	1,010	NA	NA	>67
Test suspension	4.0	>99.9	1,020	NA	NA	>67
Test suspension	4.1	99	1,090	82	103	85
Test suspension	4.1	>99.9	983	98	97	79

[a] Growth experiments utilized 25-ml serum vials containing 5 ml of phosphate-buffered medium plus 5% propane and 1.0 mg of VC liter^{-1} (0.68 μCi) and incubated for 4 weeks at 25°C. Final biomass was 200 ± 30 mg (dry weight) liter^{-1}. Inocula for cell suspensions were grown in 200 mg of tryptic soy broth liter^{-1} supplemented with 3% propane. After 5 days, cells were suspended in 5 ml of phosphate-buffered medium plus 1.0 mg of VC liter^{-1} (0.68 μCi). Vials were assayed after 7 days, and the biomass was detrmined to be 400 to 600 mg liter^{-1}.

[b] NA, Not analyzed.

vials were incubated for 4 weeks at 25°C. Visible growth ceased after 1 week. VC concentrations in inoculated controls inhibited with 0.1% Formalin and sodium azide varied less than 15% from those in uninoculated controls. Test cultures often degraded >95% of the VC during 1 month of incubation, with the average degradation among triplicates being 81, 87, and 92% during successive experiments (data not shown).

Cell suspensions grown in the presence of propane (3% [vol/vol]) or glucose (1 g liter^{-1}) also degraded VC (Table 1). The results of the experiments using glucose, acetate, yeast extract, and propane (the experiment in which the VC loss was 78% [Table 1]) were from the same experiment. Peak areas of VC in inhibited controls showed little variation, demonstrating successful retention of VC. Cells grown with acetate (0.4%) removed 53% of the VC, whereas cells grown with yeast extract plus Trypticase (BBL Microbiology Systems, Cockeysville, Md.) did not degrade VC.

To study the time course degradation of VC by cell suspensions, triplicate experimental vials and separate controls at each time point were used because VC would often rapidly escape after the Teflon-lined septa were compromised. Cells were grown for 7 days in 200 mg of tryptic soy broth liter^{-1} supplemented with propane (5% [vol/vol]), centrifuged, and suspended in 25-ml serum vials containing phosphate buffer. The biomass of the cell suspensions was 0.8 to 1.0 g (dry weight) liter^{-1}. VC loss during the initial 2 h approached 20 μmol g (dry weight)$^{-1}$ h^{-1}, after which degradation proceeded at 5 to 20 μmol of VC g (dry weight)$^{-1}$ day^{-1}. After 10-day incubations, <10% of the VC had escaped from the control vials, demonstrating retention of the volatile toxicant. Similar rates of VC loss (>5 μmol g^{-1} h^{-1}) have been reported (13, 15), but end products were not measured.

Table 2 shows mineralization of VC to carbon dioxide. Total carbon dioxide increased from 1.5% in the controls to 4% in cell suspensions and 5% in growth cultures. In all instances, VC decreased by >90%. Radioactive CO_2 was not detected from the uninoculated or inhibited controls. $^{14}CO_2$ accounted for greater than 80% of the products recovered. Soluble radioactivity increased more than 50-fold in the experimental vials, suggesting the presence of minor transformation products. Inhibited cells assimilated little radioactivity. In contrast, growing cultures and cell suspensions assimilated approximately 10% of the transformed VC into biomass. Growing cultures accumulated 7×10^6 dpm g of cells^{-1} versus 2×10^6 dpm g^{-1} in cell suspensions. These results showed that products of VC oxidation were assimilated into biomass. When soluble end products plus radioactivity assimilated into biomass were included in mass balances, an average of 85% ± 7% of the VC was transformed by isolate Sm-1 and recovered. In addition to the data presented here, transformation of [^{14}C]VC to $^{14}CO_2$ was also verified by photoionization detection-gas proportional counting.

Strain Sm-1 was similar to other chlorinated ethene-degrading microorganisms in that toluene, benzene, and vinylidine chloride could be degraded but tetrachloroethylene could not be degraded (data not shown). Interestingly, strain SM-1 could degrade VC in the absence of toluene or methane but not when grown on yeast extract plus Trypticase. Despite high volatility and poor availability in the aqueous phase, isolate Sm-1 proved capable of oxidizing VC to carbon dioxide and reducing gaseous-phase VC concentrations to less than 50 μg liter^{-1}.

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