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NOTES

Aerobic Metabolism of Trichloroethylene by a Bacterial Isolate†

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A number of soil and water samples were screened for the biological capacity to metabolize trichloroethylene. One water sample was found to contain this capacity, and a gram-negative, rod-shaped bacterium which appeared to be responsible for the metabolic activity was isolated from this sample. The isolate degraded trichloroethylene to CO_2 and unidentified, nonvolatile products. Oxygen and water from the original site of isolation were required for degradation.

The volatile chlorinated aliphatic hydrocarbons are a major concern as potential health hazards in drinking water (5). One of the most prevalent of these is trichloroethylene (TCE) (2). At present, little is known about the microbial metabolism of TCE. Partial metabolism of TCE can occur under anaerobic conditions (1, 3, 6), but the metabolites include dichloroethylenes and vinyl chloride, which are also of concern as groundwater contaminants. The only report of aerobic TCE metabolism (7) showed that mineralization of TCE occurred when soil microflora were exposed to natural gas in air, possibly implicating methanotrophs in the degradation of TCE. The present report documents the first isolation of a pure culture capable of metabolizing TCE under aerobic conditions.

A variety of soil (0.5 to 1 g suspended in 100 ml of water) and water samples from six sites in the Pensacola, Fla., area that had histories of contamination with organochlorine compounds were screened for possible TCE degradation. Samples were supplemented with concentrated stock solutions to yield a basal salts medium (4), and 5 ml aliquots were dispensed into 30-ml screw-cap culture tubes (18 by 150 mm). Tubes were sealed with Teflon-faced (E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.) neoprene rubber septa secured by hole caps to allow access by syringe. TCE (20 nmol) was added as an aqueous stock by syringe through the septum of each tube. Samples (20 µl) of the headspace from each tube were analyzed periodically by gas chromatography (Hewlett-Packard 5790 [Hewlett-Packard Co., Palo Alto, Calif.], equipped with a 30-m Supelco SPB5 capillary column [Supelco, Inc., Bellefonte, Pa.] and an electron capture detector) for changes in TCE concentration. The injector, oven, and detector temperatures on the gas chromatograph were 100, 60, and 325°C, respectively. The carrier gas was H_2 (1 ml/min) and the makeup gas was 90% argon–10% methane (45 ml/min through the detector).

Of 43 samples screened, only 1, a water sample from a holding pond at an industrial waste treatment facility for the Naval Air Station (NAS) in Pensacola, Fla., caused a substantial decrease in TCE concentration as compared to autoclaved controls. Subcultures of this sample metabolized TCE only when filter-sterilized or autoclaved water from the original sampling site (NAS water) was used to make up the

basal salts medium for the experiments, indicating that some component in the water was required for TCE metabolism. NAS water was therefore used in the medium for all subsequent tests for TCE metabolism. Samples of the active sample were plated on glucose medium (10 mM glucose, 0.05% yeast extract in basal salts medium) for isolated colonies. Resulting isolates were grown in glucose medium to stationary phase, and 1-ml portions were added to 50-ml Wheaton serum vials (Wheaton Industries, Millville, N.J.) containing NAS medium (10 ml of basal salts solution made up in NAS water and supplemented with 0.05% yeast extract). The vials were sealed with Teflon-faced neoprene serum stoppers and crimp caps, and TCE (50 nmol) was added as an aqueous stock by syringe through the septa. Changes in TCE concentrations in the medium after equilibration with the headspace of the vials were determined by extracting 1.5-ml samples with an equal volume of n-pentane and injecting 1.5 µl of the extract into the gas chromatograph under the conditions described above. All subsequent experiments also followed this method for monitoring TCE metabolism. In this manner, a pure culture, designated strain G4, which degraded TCE was eventually obtained.

Strain G4 is a nonmotile, gram-negative, rod-shaped bacterium which grows predominately in pairs and short chains in logarithmic phase. The isolate was oxidase negative, catalase positive, resistant to ampicillin and carbenicillin (greater than 100 and 1,000 µg/ml, respectively), and strictly aerobic; the isolate had no specific growth requirements.

TABLE 1. Requirement of NAS water for TCE metabolism by strain G4 and inhibition by chloramphenicol

Medium supplement	Inoculum	TCE concn (µM)[a]		% remaining
		Initial	Final	
NAS water	Active	1.35 ± 0.49	<0.02[b]	<1.5
NAS water	Autoclaved	1.28 ± 0.35	1.42 ± 0.32	110.9
NAS water, chloramphenicol (0.05 mg/ml)	Active	1.22 ± 0.20	0.91 ± 0.12	75.0
Distilled water	Active	1.22 ± 0.18	0.84 ± 0.10	68.8

[a] Each experiment was performed in triplicate; data are the means and standard deviations of three replicates. Experiments were terminated after 24 h of incubation at 26°C with shaking.
[b] Minimum detectable level.

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TABLE 2. Effect of O_2 removal on TCE metabolism by strain G4

Exptl condition[a]	Inoculum	TCE concn (μM)[b]		% remaining
		Initial	Final	
Unsparged, air headspace	Active	0.99 ± 0.10	<0.02[c]	<2
Unsparged, air headspace	Autoclaved	0.92 ± 0.03	0.81 ± 0.01	88.0
N₂ sparged	Active	1.06 ± 0.32	0.86 ± 0.32	81.1
Air sparged	Active	1.14 ± 0.01	<0.02	<1.8

[a] All experiments employed medium made with NAS water. N₂ and air sparging was performed for 30 min at 30 ml/min before addition of TCE and inoculum.

[b] Data are the means and standard deviations of three replicates. Experiments were terminated after 24 h of incubation.

[c] Minimum detectable level.

Based on these criteria, it is tentatively ascribed to the genus *Acinetobacter*.

The isolate required NAS water for complete degradation of TCE, although some disappearance of TCE also occurred in its absence (Table 1). Growth substrates for strain G4 including glucose, acetate, succinate, lactate, and ethanol would not support TCE degradation when substituted for NAS water, indicating that the component in the NAS water was not required simply for organism growth. Addition of chloramphenicol to NAS medium containing glucose-grown G4 cells prevented TCE metabolism, suggesting that the active component in the NAS water acts as an inducer for the enzyme(s) involved in TCE degradation (Table 1). In similar experiments, oxygen was required for TCE metabolism, as shown by the absence of metabolism in bottles sparged with N₂ (Table 2). Controls that were sparged with an identical amount of air showed full activity, indicating that the loss of activity after N₂ sparging was not caused by the purging of a required factor in the water.

TCE degradation was confirmed by mineralization studies. Experiments were conducted as described above, except that 37 nmol of [U-^{14}C]TCE (101,970 dpm; New England Nuclear Corp., Boston, Mass.) was added initially. After incubation for 2 days, bottles were sampled for TCE concentration, acidified with 200 μl of 2 M H_2SO_4, and sparged with N₂ at 30 ml/min for 60 min. Radiolabeled CO_2 in the effluent gas was trapped in 1 M NaOH. Radioactivity was determined by liquid scintillation (Beckman scintillation counter, model LS8200; Beckman Instruments, Inc., Fullerton, Calif.) in PCS solubilizer (Amersham Corp., Arlington Heights, Ill.) after correction for quench and background. The presence of $^{14}CO_2$ was confirmed by precipitation of the radioactivity upon addition of $BaCl_2$.

In active samples, 60% of the total ^{14}C remaining after

TABLE 3. Distribution of radioactivity after metabolism of trichloro[^{14}C]ethylene by strain G4[a]

Strain G4 inoculum	Nonvolatile products[b]		Carbon dioxide	
	dpm	% of total[c]	dpm	% of total[c]
Active	31,445 ± 1,869	34.7 ± 1.5	53,996 ± 220	59.6 ± 1.0
Autoclaved	1,785 ± 204	3.4 ± 0.5	0	0

[a] Tests were performed in triplicate, and the data are the means and standard deviations of three replicates.

[b] Radioactivity remaining in solution after acidification and sparging to remove CO_2.

[c] Based on total ^{14}C present after incubation (active = 90,552 ± 1,759 dpm; sterilized = 53,618 ± 2,329 dpm) as 100%.

incubation was CO_2, whereas no $^{14}CO_2$ was detected in autoclaved controls (Table 3). Another 35% of the total ^{14}C remaining in the active samples was unidentified, nonvolatile products, whereas only 3% was present in the autoclaved controls (Table 3). A total of 4 to 6% of the ^{14}C remaining in the active samples was present as TCE (determined by gas chromatography) and was lost during sparging for removal of $^{14}CO_2$. Passage of the medium from active samples through a 0.22-μm-pore filter removed 57 to 78% of the nonvolatile ^{14}C, demonstrating that the majority of this component was associated with particulate material and presumably incorporated into cell material. Less total ^{14}C was present in the sterile samples (53%) than in the active samples (89%) after the incubation, most likely as a result of TCE adsorption to the septum or leakage. Losses in active samples would be lower than in sterile samples because of conversion of TCE to less-volatile products.

Although strain G4 grew on a variety of substrates, including glucose, lactate, succinate, acetate, and ethanol, no growth was observed with methane (up to 50% of the culture headspace) or methanol, indicating that the organism is probably not a methanotroph and, therefore, is presumably different from the microorganisms responsible for aerobic TCE degradation in studies with soil microflora exposed to natural gas (7). In addition, strain G4 did not degrade TCE when methane or methanol was substituted for NAS water.

To our knowledge, this is the first report of the successful isolation of a pure culture that degrades TCE. The observed degradation may involve complete dechlorination since the radiolabeled carbon in the TCE appeared as CO_2 and possibly as cell biomass (radioactivity associated with particulate material). The degradation required a presently unidentified factor present in NAS water which may act as an inducer for synthesis of the enzyme(s) responsible for TCE degradation. Work is currently under way to determine the active component of NAS water and to characterize the mechanism by which strain G4 degrades TCE.

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ERRATA

Electrostatic Mechanism of Survival of Virulent *Aeromonas salmonicida* Strains in River Water

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Volume 51, no. 6, p. 1343, abstract, line 3: "virulent" should read "avirulent."

Page 1344, Table 1, column 4, lines 3 and 4: "$<10^8$" should read "$>10^8$."

Page 1344, column 1, line 18: "10 min" should read "100 min."

Page 1344, column 2, Results, line 14: "3% sodium chloride solution and within 7 days. . . ," should read "3% sodium chloride solution. Total loss of viability occurred in 5 days in distilled water or 3% sodium chloride solution, or within 7 days. . ."

Inhibitor Studies of Dissimilative Fe(III) Reduction by *Pseudomonas* sp. Strain 200 ("*Pseudomonas ferrireductans*")

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Volume 52, no. 2, p. 383: The correct version of Fig. 1 should appear as printed below.