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Degradation of Monomethylhydrazine by Two Soil Bacteria

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Ou (1987) reported that three heterotrophic soil bacteria had the capacity to degrade hydrazine. One of these organisms, Achromobacter sp., degraded hydrazine to N_2 gas. Furthermore, Ou and Street (1988) reported that monomethylhydrazine (MMH) in Arredondo fine sand was mineralized to CO_2, and that such degradation is microbial. However, microorganisms that degrade MMH have not been reported. MMH and hydrazine are chemically similar to one another. Therefore, this study was initiated to test the capacity of the two hydrazine-degrading bacteria, Achromobacter sp. and Pseudomonas sp., to degrade MMH.

MATERIALS AND METHODS

Analytical-grade MMH (99% purity) was obtained from Aldrich Chemical Company (Milwaukee, WI), and uniformly-labeled [14C]MMH with a specific activity of 6 μCi/mmol and 98% radio purity was purchased from Amersham Corp. (Arlington Heights, IL).

A strain of Achromobacter sp. and a strain of Pseudomonas sp., which had the capacity to degrade hydrazine in the presence of a second nitrogen source such as ammonium nitrate (Ou, 1987), were used for this study. Both bacteria were isolated from Arredondo fine sand. The bacteria were maintained, as described previously (Ou, 1987) in a basal mineral medium containing 10 μg/g of MMH or 100 μg/g of hydrazine.

MMH in culture fluids and cell suspensions was determined by the colorimetric method of Reynolds and Thomas (1965). MMH in soil samples was extracted with cold and deoxygenated 0.1M HCl as described previously (Ou and Street, 1988), and then determined by the colorimetric method of Reynolds and Thomas (1965). The key properties of Arredondo fine sand, used for this study, have previously been reported (Ou and Street, 1987). In addition, [14C]MMH was used for determination of MMH disappearance and formation of metabolites. Radioactivity in culture fluids was determined by scintillation counting. [14C]metabolites were

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determined by organic-solvent extraction, thin-layer chromatography (TLC)-autoradiography, and scintillation counting. Radioactivity in culture fluids was determined by extracting with an equal volume of chloroform and ethyl ether. The organic extracts, after removing moisture with anhydrous sodium sulfate, were concentrated to 1.5-2.0 mL in a stream of N_2 gas. Aliquots (10 to 20 µL) of the extracts were spotted on commercial silica gel G plates. The TLC developing-solvent system was hexane-chloroform-methanol (7:2:1, v/v). Radioactive areas on each plate were detected by placing Kodak SB-5 X-ray films on the plates. The radioactive areas on the plates were scraped, transferred to scintillation vials, and quantified by scintillation counting.

[14C]CO_2 evolved from growing cultures in glass Erlenmeyer flasks containing [14C]MMH was trapped in small stainless steel vials containing 1 g of KOH pellets. The vials were hung under the rubber stoppers in the flasks using stainless steel wire. The flasks were then tightly closed with the stoppers. After removing the KOH from the flasks it was diluted with water to 4 mL, and radioactivity in the KOH solutions was determined by scintillation counting.

Growth of bacterial cultures was determined turbidimetrically at 550 nm with a Spectron 20 spectrophotometer.

Sixteen- to twenty-hour-old cultures were harvested by centrifugation in the cold, washed once with cold phosphate buffer (pH 7.2), and resuspended in the same buffer.

All bacterial cultures and suspensions were incubated at 25°C. All experiments were carried out in duplicate.

RESULTS AND DISCUSSION

MMH at 25 and 100 µg/mL was rapidly degraded by the growing cultures of both Achromobacter sp. and Pseudomonas sp. (Figures 1 and 2). Both bacteria required a second carbon source (glucose) and a second nitrogen source (ammonium nitrate) for growth. Since only small amounts of MMH were degraded in the autoclaved cultures, degradation of MMH in the growing cultures was principally microbial. MMH at both concentrations was rapidly degraded by the growing culture of Pseudomonas sp. without a lag period. This was possibly due to high initial inoculum level and/or to high initial degradative enzyme activity. Higher MMH concentrations appeared to prolong the lag phase of bacterial growth. Although the Achromobacter sp. degraded MMH, growth of this organism in the presence of MMH at the end of the incubation period (96 hours) had not reached the same level as in the absence of MMH.

Neither Achromobacter sp. nor Pseudomonas sp. degraded MMH to CO_2. Although MMH was completely degraded in 4 days by both bacteria, the majority of the applied [14C] still remained in the culture fluids (Table 1). Less than 4% of the applied [14C] was found in

Table 1. Radioactivity in culture fluids of Achromobacter sp. and Pseudomonas sp., and in KOH traps after 4 days of incubation.

MMH (µg/g)	% of Applied [14C]		
	[14C] Remaining in Culture Fluids	[14C] Trapped in KOH	Recovery
		Achromobacter sp.	
25	94.4	4.8	99.2
100	94.0	4.7	98.7
		Pseudomonas sp.	
25	96.4	4.4	100.8
100	96.8	4.4	101.2

Table 2. Degradation of MMH in live and autoclaved cell suspensions of Achromobacter sp. and Pseudomonas sp.[a]

Cell Suspension	Initial MMH (µg/g)	% Reduction
	Achromobactar sp.	
Live[b]	22	100
Live[b]	41	100
Live[b]	81	83
Live[b]	117	57
Autoclaved[c]	21	3
Autoclaved[c]	64	3
	Pseudomonas sp.	
Live[d]	21	100
Live[d]	45	96
Live[d]	86	63
Live[d]	117	34
Autoclaved[e]	22	4
Autoclaved[e]	67	6

[a]Cell suspensions were incubated at 25°C for 1 hour.
[b]Cell dry weight 4.0 mg/mL.
[c]Cell dry weight 4.0 mg/mL.
[d]Cell dry weight 4.1 mg/mL.
[e]Cell dry weight 4.3 mg/mL.

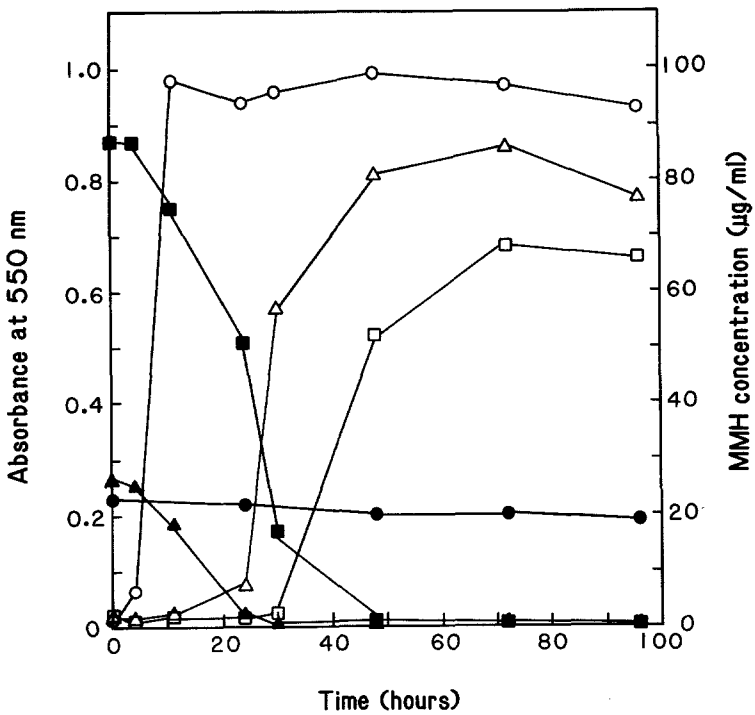

Figure 1. MMH degradation and growth of Achromobacter sp. Designations: ○, △, and □, absorbance of culture fluids with initial MMH concentrations of 0, 27 and 88 µg/mL, respectively; ▲ and ■, MMH concentrations in culture fluids; and ●, MMH concentration in the culture-free medium.

the KOH traps. No change of radioactivity in the KOH traps was observed before and after acidification of the KOH. This indicated that the trapped [14C] was not associated with $[^{14}C]CO_2$. The trapped [14C] probably consisted of [14C]MMH and volatile metabolite(s). MMH is somewhat more volatile than water (Schmidt, 1984). Total [14C] recoveries for all treatments were near 100%. TLC-autoradiographic assays confirmed the results of the colorimetric determinations, that MMH had completely disappeared from 4-day-old cultures of the two bacteria. The TLC-autoradiographic assays also revealed that MMH was degraded to water-soluble polar metabolite(s) (R_f value = 0).

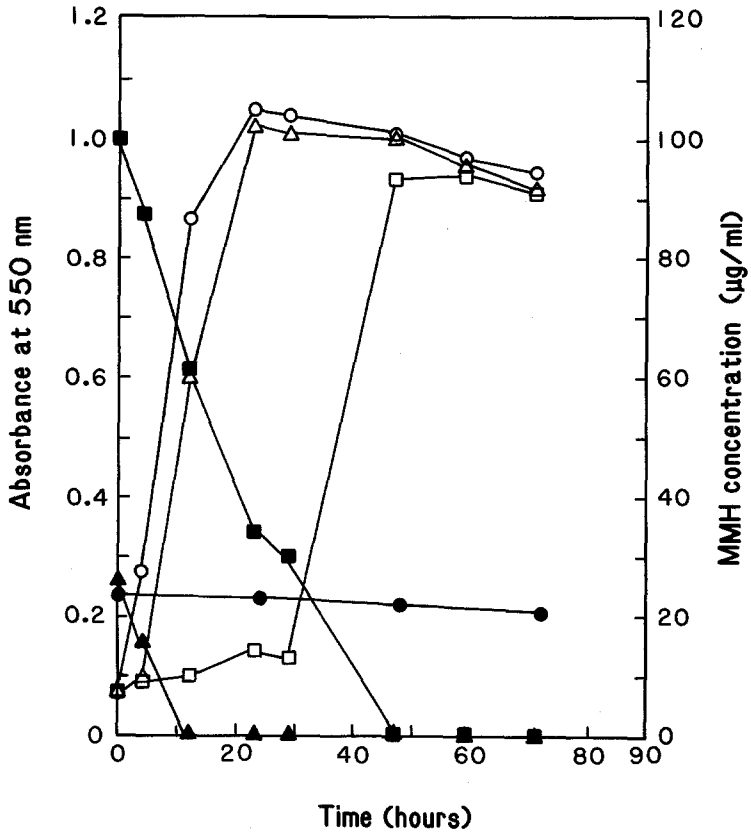

Figure 2. MMH degradation and growth of Pseudomonas sp. Designations: ○, △, and □, absorbance of culture fluids with initial MMH concentrations of 0, 27 and 100 µg/mL, respectively; ▲ and ■, MMH concentrations in culture fluids; and ●, MMH concentration in the culture-free medium.

Resting cell suspensions of the two organisms had a high capacity to degrade MMH (Table 2). During 1 hour of incubation, MMH at concentrations under 50 µg/g was either completely or near-completely degraded. Even at 117 µg/g, 57 and 34% of the MMH were degraded in cell suspensions of the Achromobacter sp. and the Pseudomonas sp., respectively. Less than 6% of the MMH disappeared from autoclaved cell suspensions. This suggests that degradation in the resting-cell suspensions was principally microbial. Since a substantial amount of [14C]MMH in soil is mineralized to $[14C]CO_2$ (Ou and Street, 1988), and since such degradation is microbial, microorganisms that utilize MMH as a sole source of carbon for growth may exist. Efforts are being made to isolate such microorganisms from soil.

Table 3. Degradation of MMH in Arredondo soil inoculated with live and dead cells of Achromobacter sp. and Pseudomonas sp.[a]

Bacteria	MMH (µg/g)	% Reduction
	Nonsterile soil	
Achromobacter sp., live[b]	90	55
Achromobacter sp., dead[c]	140	30
Pseudomonas sp., live[d]	112	44
Pseudomonas sp., dead[e]	140	30
	Autoclaved soil	
Achromobacter sp., live[b]	54	73
Achromobacter sp., dead[c]	153	23
Pseudomonas sp., live[d]	120	40
Pseudomonas sp., dead[e]	176	12

[a]Soil samples were incubated at 25°C for 1 hour, and the initial MMH concentration in soil was 200 µg/g.
[b]Cell dry weight 0.8 mg/g.
[c]Cell dry weight 0.8 mg/g.
[d]Cell dry weight 0.9 mg/g.
[e]Cell dry weight 0.9 mg/g.

Both Achromobacter sp. and Pseudomonas sp. accelerated the degradation of MMH in nonsterile and autoclaved Arredondo soil (Table 3). More MMH was degraded in soil samples inoculated with live bacterial cells than in soil samples inoculated with dead bacterial cells. The Achromobacter sp. was more active in degrading MMH, especially in autoclaved soil, than the Pseudomonas sp. More MMH was degraded in the nonsterile soil samples inoculated with dead cells than in the autoclaved soil samples inoculated with dead cells. This indicated that indigenous microorganisms, in addition to autooxidation, were also involved in the degradation of MMH. But the degradative capacity for the native microbial communities was not as great as the Achromobacter sp. or the Pseudomonas sp.

In conclusion, two hydrazine-degrading soil bacteria Achromobacter sp. and Pseudomonas sp. were demonstrated to have the capacity to degrade MMH in culture media and soil samples. Despite the fact that Achromobacter sp. and Pseudomonas sp. could not utilize MMH as a sole source of carbon for growth and did not degrade MMH to its final oxidation products, CO_2 and H_2O, the organisms may have potential for use in the detoxification of hydrazine- and MMH-contaminated soils, water, and wastes. In this case, the nature of the water soluble polar metabolite(s) needs to be determined.

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