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Microbial Degradation of Hydrazine

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Hydrazine, which is the simplest form of diamines, is produced in large quantity annually for use in aerospace, power plant, and agricultural and pharmaceutical applications (Schmidt, 1984). These and other uses have led to concern about the fate of this chemical and its derivatives in the environment. Hydrazine, when present in natural waters (river, lake and pond) in small quantities, was not stable (Solonium and Gisclard, 1976). Hydrazine in soil was even much less stable. Ou and Street (1987) reported that hydrazine applied to Arredondo soil at concentrations of 100 and 500 µg/g completely disappeared in less than 1 and 8 d, respectively. By comparing degradation rates in sterile and nonsterile soils, they concluded that biological degradation was responsible for about 20% of the disappearance of the chemical.

Hydrazine is toxic to many forms of bacteria. The activities of the autotrophic nitrifiers Nitrosomonas and Nitrobacter, denitrifying bacteria, and anaerobic methanogens were inhibited by the chemical (Kane and Williamson, 1983). In addition, hydrazine prolonged the lag phase of growth as well as inhibited growth of the soil bacterium Enterobacter cloacae (London and Mantel, 1983; London et al., 1983; Mantel and London, 1980). Due to rapid disappearance in soil, hydrazine at 100 µg/g exhibited only temporary inhibition on soil bacterial populations (Ou and Street, 1987). However, hydrazine at 500 µg/g caused a significant reduction of soil bacterial populations, although fungal populations in soil were enhanced by the presence of hydrazine. Despite its toxicity to soil bacteria, Kane and Williamson (1983) demonstrated that hydrazine in small quantities was cometabolically degraded to nitrogen gas by Nitrosomonas. An enzyme system of nitrogen-fixing heterotrophic bacteria was able to convert hydrazine to ammonia (Stiefel et al., 1977). However, it was not clear that the bacteria could utilize hydrazine as a sole source of nitrogen for growth.

In the present study, I describe the isolation of an Achromobacter sp. from soil that had a high capacity to degrade hydrazine. Attempts to establish degradation pathways are also reported.

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MATERIALS AND METHODS

Hydrazine sulfate and hydrazine monohydrate were purchased from Aldrich Chemical Company (Milwaukee, WI). Hydrazine sulfate was used in all experiments unless otherwise specified. Uniformly labeled ^{15}N-hydrazine sulfate was obtained from Icon (Summit, NJ), with the chemical consisting of 98 atom % of ^{15}N.

Basal mineral medium consisted of (per liter of distilled water) 4.8 g of K_2HPO_4, 1.2 g of KH_2PO_4, 1 g of NH_4NO_3, 0.25 g of $MgSO_4 \cdot 7H_2O$, 0.04 g of $CaCl_2$, and 0.001 g of $Fe_2(SO_4)_3$. Glucose, 0.2 g/ml, and hydrazine sulfate, 0.01 g/ml, were sterilized separately by autoclaving and filtration, respectively. For maintenance of bacterial cultures, ten ml of the glucose and ten ml of the hydrazine were added to 1 L of the basal mineral medium. Tryptic soy agar and broth (Difico) were also used for purification and maintenance of bacterial cultures.

One hundred g of Arredondo fine sand (Grossarenic Paleudult) were repeatedly treated with 100 µg/g of hydrazine. This soil had previously been shown to readily degrade hydrazine at 100 µg/g (Ou and Street, 1987). After four applications, 10 g of the soil were transferred to a culture tube containing 10 ml sterile distilled water. After the tube was briefly shaken, 0.3 ml aliquots of the suspension were spread on NH_4NO_3-free basal mineral agar plates supplemented with glucose and hydrazine. The plates were incubated at 25 C for observation of the development of bacterial and fungal colonies. Bacterial and fungal colonies of different appearance were further transferred to new plates. The bacterial isolates were also transferred to tryptic soy agar plates for purification.

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

Eighteen-h old cultures were harvested by centrifugation at 20,000xg for 15 min at 4 C, washed once with potassium phosphate buffer (K_2HPO_4, 4.8 g/L and KH_2PO_4, 1.2 g/L), and resuspended in the same buffer.

Frozen cells (3 g) were suspended in 6 ml of the potassium phosphate buffer. The suspension was irradiated with ultrasonic waves for 9 min with an ultrasonic cell disruptor (Sonified W140, Heat Systems-Ultrasonic), using a titanium probe at 20,000 Hz and 2 A. The resulting suspension was centrifuged at 30,000xg for 30 min. The clear supernatant was used as a source of crude cell extracts. Protein concentration in cell extracts was determined by the method of Bradford (1976), using bovine serum albumin as the standard.

Hydrazine was colorimetrically determined using the method of Watt and Chrisp (1952). 0.01-0.2 ml of supernatants from cell cultures, cell suspensions, or cell extracts was transferred to 25 ml

of volumetric flasks which had contained 10 ml of color-developing agent, p-dimethylaminobenzaldehyde, and sufficient 1 M HCl was added to make a total volume of 25 ml. Optical densities of the samples were determined at 458 nm using a Spectronic 20 spectrophotometer.

Ammonia concentrations in cell suspensions were determined by a specific ammonia electrode as described previously (Ou and Street, 1987).

Nitrate concentrations in cell suspensions were determined by a nitrate-specific electrode (Ou and Street, 1987). Nitrate concentrations in cell suspensions were determined colorimetrically at 520 nm using the method of Barnes and Folkard (1984).

$^{15}N_2$ gas produced in cell suspensions treated with ^{15}N-hydrazine sulfate was determined by mass spectometry. 6 ml aliquots of cell suspension were placed in 20 ml glass serum bottles which contained 0.6 or 0.9 mg of ^{15}N-hydrazine. 0.2 ml of air in the headspace was withdrawn for mass spectral analysis.

RESULTS AND DISCUSSION

Fungal colonies were found to develop in the NH_4NO_3-free basal mineral agar plates supplemented with glucose and hydrazine. However, they either did not grow or grew poorly in liquid basal mineral medium supplemented with hydrazine and glucose with or without NH_4NO_3. The fungi were found to have little capacity to degrade hydrazine. Hence, they were not used for further investigation. Bacterial colonies of small size developed a few days after fungal colonies appeared. The bacteria all failed to grow in liquid NH_4NO_3-free basal mineral medium supplemented with glucose and hydrazine. However, some of the bacteria grew when supplemented with NH_4NO_3. An Achromobacter sp., a Bacillus sp., and a Pseudomonas sp. were found to have capacity to degrade hydrazine. Both Bacillus sp. and Pseudomonas sp., when grown in glucose-amended basal mineral medium containing hydrazine, had a lag growth period of 3 to 5 days. The cell suspensions of the two bacteria could only degrade hydrazine at concentrations of 25 µg/ml and lower. The Achromobacter sp. not only had a short lag growth period but also degraded hydrazine at concentrations greater than 100 µg/ml. Therefore, this bacterium was chosen for further study.

The Achromobacter sp. in glucose-amended basal mineral medium containing 25 and 51 µg/ml hydrazine exhibited 4 and 8 h of lag growth period, respectively (Figure 1). The bacterial culture at both hydrazine concentrations reached maximal growth by 48 h. Unlike the bacterial growth, hydrazine was degraded without a lag period. Degradation had levelled off before cell growth reached maximal.

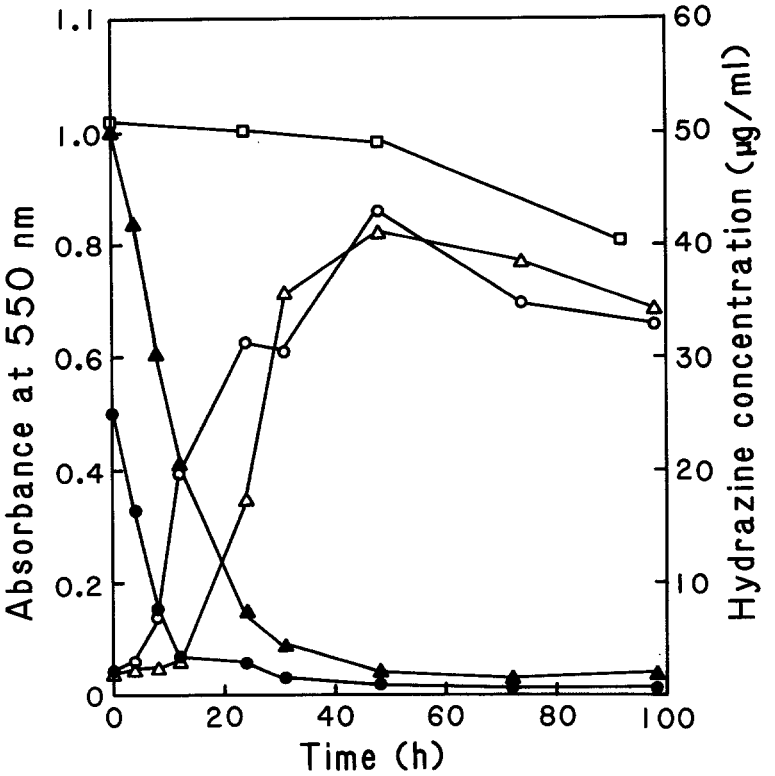

Figure 1. Hydrazine degradation and growth of the Achromobacter sp. Designations: O and △, absorbance of culture fluids with initial hydrazine concentrations of 25 and 51 µg/ml, respectively; ● and ▲, hydrazine concentrations in culture fluid; and □, hydrazine concentrations in the culture-free glucose-amended medium.

Hydrazine at 51 µg/ml in the glucose-amended basal mineral medium in the absence of the bacterial culture was much more stable than in the presence of the culture (Figure 1). After 48 h of incubation 97% of hydrazine remained in the culture-free medium, whereas only 4% of the chemical remained in the culture medium during the same incubation period. In addition, hydrazine at 25 µg/ml was also stable in the culture-free glucose-amended basal mineral medium. Hydrazine was also found to be stable in the basal mineral medium, in a glucose solution (0.2 g/ml), and in distilled water.

Hydrazine, at various concentrations ranging from 25 to 162 µg/ml, was used for testing the degradative capacity of cell suspensions of the Achromobacter sp. which had been grown in hydrazine and glucose-amended basal mineral medium. Table 1 shows that hydrazine at initial concentrations of 25, 50 and 90 µg/ml was degraded

Table 1. Hydrazine in cell suspensions of the Achromobacter sp. grown in basal mineral medium containing hydrazine[a]

Cell suspension	Initial N_2H_4 (µg/ml)	% Reduction
Live[b]	25	96
Live[b]	50	95
Live[b]	90	94
Live[b]	104	84
Live[b]	120	56
Live[b]	162	52
Heated[c]	50	4
Autoclaved[d]	50	0

[a] Cell suspensions were incubated at 25 C for 2 h.
[b] Dry cell weight of the suspension was 3.8 mg/ml.
[c] Cell suspension was heated in a waterbath at 90 C for 10 min.
[d] Cell suspension was autoclaved at 121 C for 15 min.

to near completion within 2 h, and at 120 and 162 µg/ml more than 50% of the chemical was degraded. Hydrazine in heated and autoclaved suspensions was stable and, in fact, no hydrazine in the autoclaved suspension was degraded during 2 h of incubation.

Nitrate, nitrite and ammonia were not formed in cell suspensions treated with hydrazine. $^{15}N_2$ gas was detected in air samples from the headspace of serum bottles containing the bacterial cell suspension and ^{15}N-hydrazine. ^{15}N-hydrazine was not detected in the air samples.

The cell suspension of the Achromobacter sp. which had been grown in the basal mineral medium without hydrazine also had a capacity to degrade hydrazine (Table 2). The bacterium grown in the basal mineral medium with KNO_3 or $(NH_4)_2SO_4$ as a sole source of nitrogen also degraded hydrazine. Furthermore, bacterial cells grown in rich complex medium such as tryptic soy broth also had a capacity to degrade hydrazine.

The Achromobacter sp. also degraded the nonsalt form of hydrazine. It was found that only 6% of applied hydrazine monohydrate (25 µg/ml) remained in the cell suspension after 2 h of incubation. Autoclaved cells lost their capacity to degrade hydrazine monohydrate.

Crude cell extracts of the Achromobacter sp. also had a capacity to degrade hydrazine. The specific activity of the extracts toward the reduction of hdyrazine was estimated to be 0.30 µmol/h/mg protein. Heated extracts lost their capacity to degrade hydrazine. Hydrazine in phosphate buffer was not degraded.

These results suggest that the enzyme system for the metabolism of hydrazine by the Achromobacter sp. is constitutive. This is evidenced by the fact that hydrazine is rapidly degraded without a

Table 2. Degradation of hydrazine in cell suspensions of the Achromobacter sp. grown in media without hydrazine[a]

Cell suspension	Initial N_2H_4 (µg/ml)	% Reduction
Grown in glucose - amended basal mineral medium[b]	42	94
Grown in glucose - amended basal in mineral medium[b]	83	86
Grown in glucose - amended basal mineral medium[c]	27	93
Grown in glucose- amended basal mineral medium[d]	27	97
Grown in tryptic soy broth[e]	50	86
Grown in tryptic soy broth[e]	125	83

[a] Cell suspensions were incubated at 25 C for 2 h.
[b] Cell dry weight 3.7 mg/ml. Nitrogen source in the basal mineral medium was NH_4NO_3.
[c] Cell dry weight 3.6 mg/ml. Nitrogen source in the basal mineral was KNO_3.
[d] Cell dry weight 3.8 mg/ml. Nitrogen source in the basal mineral was $(NH_4)_2SO_4$.
[e] Cell dry weight 4.0 mg/ml.

lag. The failure of autoclaved and heated cells, and of heated cell extracts, to degrade the chemical indicates that degradation during short incubation is principally microbial. For long incubations, autooxidation may play a role as well (Schmidt, 1984). Under the systems used in this study, the role of autooxidation is negligible.

Similar to the growth response of the soil bacterium Enterobacter cloacae to hydrazine (London and Mantel, 1983; Mantel and London, 1980), an increase in hydrazine concentration caused an extension of the lag growth period for Achromobacter sp. However, lag growth periods were short. This is because more than 50% of the hydrazine has already been degraded at the onset of exponential growth. Obviously the enzymes responsible for degradation of the chemical are not poisoned or deactivated by the chemical.

Inability of the Achromobacter sp. to grow on hydrazine as a sole source of nitrogen indicates that the metabolic process for hydrazine is cometabolic. The Achromobacter sp. utilizes nitrate or ammonia as a sole source of nitrogen for growth, and these cells degrade hydrazine. Thus, it is likely that some common enzymes responsible for metabolism of nitrate and ammonia also have a capacity to degrade hydrazine. Oxidation of hydrazine to nitrogen gas by Nitrosomonas sp. has also been suggested to be a cometabolic process (Kand and Williams, 1983).

It is understandable that nitrate, nitrite and ammonia were not the degradation products of hydrazine. If those products had been formed Achromobacter sp. should be able to utilize hydrazine for growth. Likewise, it is also unlikely that hydroxylamine is a degradation product. Hydroxylamine is toxic and unstable (Alexander, 1977; Yoshida and Alexander, 1964), and is suspected to be an intermediate of nitrate metabolism (1).

The observation that some common heterotrophic soil bacteria, such as Achromobacter sp., Bacillus sp. and Pseudomonas sp., as well as autotrophic nitrifiers reported by Kane and Williamson (1983), can degrade hydrazine suggests that microbial degradation, in addition to autooxidation, may play a role in removing this chemical from the environment. In light of the rapid degradation by Achromobacter sp. which was observed at concentrations beyond 100 µg/ml, the bacterium may have potential for use in the detoxication of hydrazine from contaminated soils and waters, and from wastes.

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REFERENCES

Alexander M (1977) Introduction to soil microbiology. John Wiley & Sons, New York.

Barnes H, Folkard AR (1951) The determination of nitrites. Analyst 76: 599-603.

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72: 248-554.

Kane DA, Williamson KJ (1983) Bacterial toxicity and metabolism of hydrazine fuels. Arch Environ Contam Toxicol 12: 447-453.

London SA, Mantel CR (1983) Effect of hydrazines on substrate utilization by a strain of Enterobacter cloacae. Bull Environ Contam Toxicol 30: 497-504.

London SA, Mantel CR, Robinson JD, Luking S (1983) Effects of selected hydrazines on the early death rates of Enterobacter cloacae. Bull Environ Contam Toxicol 31: 360-368.

Mantel C, London S (1980) Adaptation of a soil bacterium to hydrazine propellants. Bull Environ Contam Toxicol 25: 762-770.

Ou LT, Street JJ (1987) Hydrazine degradation and its effect on microbial activity in soil. Bull Environ Contam Toxicol. 38: 179-183.

Schmidt EW (1984) Hydrazine and its derivatives. John Wiley & Sons, New York.

Slonium AR, Gisclard JB (1976) Hydrazine degradation in aquatic systems. Bull Environ Contam Toxicol 16: 301-309.

Stiefel EI, Newton WE, Watt GD, Hadfield KL, Bulen WA (1977) Molybdoenzymes: The role of electrons, protons, and dihydrogen. Adv Chem Series 162: 353-388.
Watt GW, Crisp, JD (1952) A spectrophotometric method for determination of hydrazine. Anal. Chem. 24: 2006-2008.
Yoshida T, Alexander M (1964) Hydroxylamine formation by Nitrosomonas europaea. Can J Microbiol 10: 923-926.
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