Mineralization of the Herbicide Atrazine in Soil Inoculated with a *Pseudomonas* Strain

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The *Pseudomonas* strain YAYA6 growing on atrazine as carbon source in liquid media retained over 50% of its atrazine degrading activity determined in liquid medium after inoculation into a nonsterile soil. Evidence is given that the bacteria grow in nonsterile soil using atrazine as a substrate. Atrazine was rapidly degraded in inoculated soil to concentrations below 0.1 mg/kg, and the results show that concentrations below 0.01 mg/kg can be reached. As in liquid medium, atrazine was mineralized in inoculated nonsterile soil with liberation of $^{14}CO_2$ from U-*ring*-^{14}C-labeled atrazine. Mineralization proceeded to over 60% when the concentration of atrazine had dropped to less than 1% of its initial concentration. Atrazine biodegradation by bacteria of the strain YAYA6 in soil was less efficient at reduced soil water contents, under conditions of limited oxygen supply, and in a soil with a pH below 7. In a soil with high organic matter content and in a soil that had been preincubated with atrazine prior to the addition of the bacteria, the lower bioavailability limited the rate of atrazine biodegradation by strain YAYA6. The bacteria retained a substantial part of their atrazine degrading activity and probably survive during a 6 week incubation period in nonsterile soils without atrazine.

Keywords: *Atrazine; mineralization; bacteria; Pseudomonas; soil*

INTRODUCTION

Degradation of the herbicide atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine] (AT) in soil has been studied by numerous workers. The average half-life in agricultural soil is around 40 days (Seiler et al., 1992), and the main product is deethylatrazine (Erickson and Lee, 1989). Mineralization in soils proceeds slowly. Wolf and Martin (1975) recovered 20% of the (*ring*-^{14}C)AT applied onto soil as $^{14}CO_2$ after 2 years of incubation. Similar data were obtained by Nair and Schnoor (1994). McMahon et al. (1992) recovered around 1% $^{14}CO_2$ from (*ethyl*-2-^{14}C)AT per day but less than 0.1% from (U-*ring*-^{14}C)AT over 23 days. Behki and Khan (1994) showed that *Rhodococcus* strain B-30 isolated from a soil treated repeatedly with *S*-ethyl dipropylthiocarbamate metabolizes AT in an aqueous medium within 72 h, with formation of deethylatrazine and deisopropylatrazine. Mandelbaum et al. (1995) have isolated a strain of *Pseudomonas* sp. using AT as nitrogen source and mineralizing it via the formation of hydroxyatrazine. By addition of this strain to soil, AT degradation could be slightly increased.

The strain YAYA6 used in this study has been isolated from a mixed microbial community using AT as sole carbon source (Gschwind, 1992, 1993). Cells of strain YAYA6 also grow on AT as sole carbon source. In aqueous media AT is degraded efficiently between pH 7 and 9 via dechlorination and formation of hydroxyatrazine and via N-dealkylation and formation of deethylatrazine or deisopropylatrazine (Yanze Kontchou and Gschwind, 1994). Around 50% of the label in (U-*ring*-^{14}C)AT was recovered as $^{14}CO_2$ after 7 weeks of incubation, and cyanuric acid was found to be the major *s*-triazine metabolite. From a starting AT concentration of 30 mg/L, end concentrations around 0.1 μg/L were reached.

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Table 1. Characteristics of Soil Samples

	soil samples		
	Moehlin	Bellechasse	Schafisheim
pH	7.2	7.5	5.4
organic matter (%)	3.7	36	2.0
sand (%)	31	na[b]	56
silt (%)	37	na	30
clay (%)	31	na	13
biomass (μg of C/g)	520	na	450
K_d[a]	1.6	20.0	1.3

[a] Linear distribution coefficient between water and soil. [b] na, not analyzed.

The purpose of this study was to determine whether and under which conditions strain YAYA6 degrades the herbicide AT in soil. Preliminary results have been given by Yanze Kontchou and Gschwind (1993).

MATERIALS AND METHODS

Chemicals and Soil Characteristics. AT of 99% purity and (U-*ring*-^{14}C)AT with a specific activity 1.27 kBeq/mg were used in our assays.

Three soil samples from Switzerland, air-dried and sieved through 2 mm mesh with the characteristics listed in Table 1, were used in this work. Adsorption coefficients (K_d) were determined by shaking 2 ppm AT in a 1:2 soil/water suspension overnight and analyzing the equilibrum AT concentration in the aqueous phase by HPLC. Soil K_d values given are the linear adsorption coefficients (i.e. concentration in soil/concentration in aqueous phase at equilibrium).

Culture Conditions. YAYA6 was grown in mineral–AT–glucose medium under aerobic conditions (Yanze Kontchou and Gschwind, 1994). Cell density was monitored by measuring the turbidity at 600 nm in a 1 cm cuvette on a UV–vis spectrophotometer. An A_{600} of 1.0 corresponds to 2.10^{10} CFU/mL determined on a mineral medium containing 17 g/L agar, 200 mg/L glucose, 20 mg/L yeast extract, and 80 mg/L atrazine. For the inoculation of soil samples, cells were collected by centrifugation (10000*g* for 20 min) and resuspended in phosphate buffer to obtain an A_{600} of 1.35.

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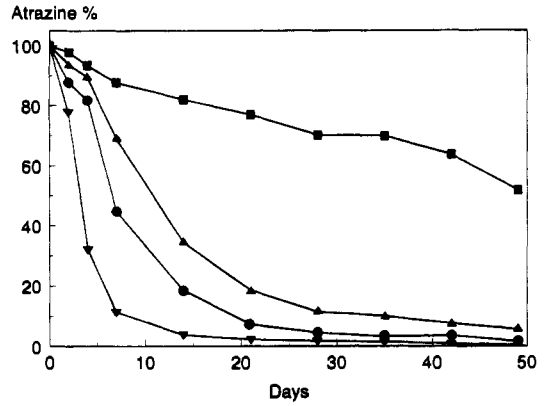

Figure 1. Degradation of AT in Moehlin soil. The AT concentration in percent of the initial concentration is shown for noninoculated control soil (■), soil inoculated with strain YAYA6 (▼), soil with 5-fold diluted inoculum (●) and soil with 25-fold diluted inoculum (▲). The data points shown are mean values from duplicate samples.

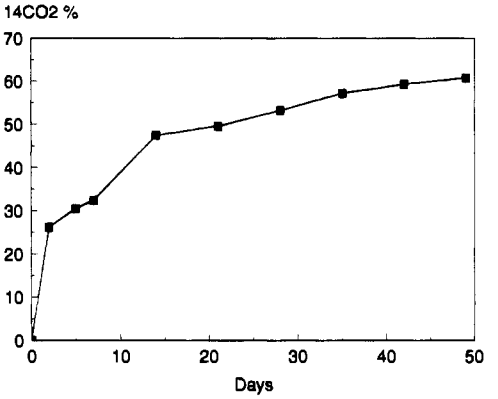

Figure 2. Liberation of $^{14}CO_2$ from Moehlin soil with (U-*ring*-^{14}C)AT after inoculation with strain YAYA6. The results are given in percent of the total radioactivity applied to the soil samples. The data points shown are mean values from triplicate samples. Less than 0.1% of $^{14}CO_2$ was liberated from noninoculated control soil.

Biodegradation Tests. Tests were performed in 60 mL glass vials containing 20 g of air-dried soil with AT at a concentration of 10 mg/kg of dry soil (200 μg per vial). The inoculum (1 mL if not specified otherwise) and distilled water were added to obtain a water content of 22%. The vials were stoppered, homogenized by shaking vigorously, and incubated in the dark at 22 °C. At different times, three replicate vials were removed and stored at −20 °C until the time of analysis. The vials were opened weekly and exposed to the atmosphere for 15 min, closed, and homogenized again. Degradation half-lives were calculated by linear regression of the log of the remaining AT concentration against time. The standard deviation among the three replicates was less than 15% in all cases.

For mineralization studies using (U-*ring*-^{14}C)AT, 40 g of Moehlin soil with 0.4 mg of labeled AT was incubated in 100 mL rubber-stoppered serum bottles at 30 °C. $^{14}CO_2$ was trapped in 2 mL of 0.25 M KOH solution contained in cups inside the serum bottles. At the end of the incubation period the soil was acidified to pH 2 by addition of H_3PO_4, and the $^{14}CO_2$ was assayed by liquid scintillation spectrometry (BETAmatic II, Kontron Instruments, Zürich, Switzerland).

HPLC Analysis. AT was extracted from soil samples by adding 20 mL of acetonitrile (Gradient grade, Merck) to each vial and shaking vigorously for 2 h on a rotatory shaker at room temperature. The supernatant was filtered through a Millex HA 0.2 μm size filter. Samples of 20 μL were analyzed on a Hewlett-Packard 1084 B liquid chromatograph with a 125 × 4 mm Nucleosil 100 C_{18} 5 μm reversed phase column with a 20 mm precolumn and a UV detector at 220 nm. A mixture of 40% acetonitrile Gradient grade and 60% LiChrosolv water (Merck) at a flow rate of 1 mL/min was used for elution. Extraction efficiencies at 1, 5, and 10 mg/kg were determined in all soils used at different times of the experiments and were always found to be above 99%.

RESULTS AND DISCUSSION

Biodegradation of AT in Moehlin Soil. Figure 1 shows the disappearance of AT incubated at 22 °C in uninoculated Moehlin soil and in Moehlin soil inoculated with different dilutions of the same cell suspension of strain YAYA6. The experiment was run at 20% moisture content, which corresponds to ca. 50% of the water holding capacity. The first-order degradation half-lives were calculated as 57 days for the control, 3.4 days for the soil inoculated with the undiluted cell suspension, and 6.0 and 11 days for the soils inoculated with the 5- and the 25-fold diluted inocula. The inocula used for these experiments were at an activity of 50 μg of AT degraded per milliliter and per day. The mean half-life of AT in the soil inoculated with 1 mL of the undiluted inoculum indicates that the cells retained about 60% of their AT degrading activity determined in an aqueous medium without taking into account growth during incubation in soil. From the growth yield of 80 g of dry cells per mole of AT in liquid medium (Yanze Kontchou and Gschwind, 1994) it can be estimated that the amount of AT present in the soil allows a 3-fold increase in biomass in the case of the undiluted inoculum. In the case of the 5- and 25-fold diluted inoculum the influence of growth during the test is more pronounced and the half-lives do not increase by a factor of 5 as expected with nongrowing cells.

The mean end concentrations measured after 7 weeks of incubation were 0.03 mg/kg for the undiluted inoculum, 0.15 mg/kg for the 5-fold diluted inoculum, and 0.56 mg/kg for the 25-fold diluted inoculum. As AT concentrations were decreasing in all cases between 42 and 49 days, AT concentrations below 0.03 mg/kg can be obtained upon prolonged incubation.

To prove mineralization, (U-*ring*-^{14}C)AT was incubated in Moehlin soil at 30 °C and 50% of the water holding capacity. The inocula used were at an activity of 200 μg of AT degraded per day per bottle. From the results shown in Figure 1 the half-life of AT under these conditions can be estimated to be around 1 day. Figure 2 shows the production of $^{14}CO_2$: 47% of the radioactivity was recovered as $^{14}CO_2$ within 14 days and 61% after 49 days of incubation. Less than 0.1% of $^{14}CO_2$ was formed in uninoculated controls. As in liquid medium (Yanze Kontchou and Gschwind, 1994) fission of the triazine ring proceeds when the concentration of AT has dropped below 1% of the value at the start of the experiment.

Influence of Soil Conditions. The effect of soil water content on atrazine degradation in Moehlin soil inoculated by YAYA6 was studied. Using an identical inoculum and incubation at 22 °C, a half-life of 0.9 day was obtained at 20% water content (50% of the water holding capacity). The half-life rose to 2.0 days at 10% water content and to 13.5 days at 5% water content. These data are in agreement with the results given by Nair and Schnoor (1994), who found an 8-fold decrease of the rate of AT mineralization in soil when the soil water content was reduced from 30 to 15% of the field capacity.

When degradation experiments were performed using Moehlin soil in test vials flushed with nitrogen, the half-

life of AT increased by a factor of 2 compared to the aerated controls. This indicates that the strain YAYA6 degrades AT in soil under conditions of restricted oxygen supply. Stucki et al. (1995) showed that the mixed microbial community from which strain YAYA6 was isolated is able to mineralize AT under carbon-limited conditions in the absence of molecular oxygen using nitrate as electron acceptor.

The influence of a high soil organic matter content was studied by comparing AT degradation in Moehlin soil and in Bellechasse soil (Table 1) under the same experimental conditions. The half-life of AT in uninoculated controls was 55 days in Moehlin soil and 54 days in Bellechasse soil. After inoculation with strain YAYA6, the half-lives were found to be 1 day in Moehlin and 22 days in Bellechasse soil. The K_d values given in Table 1 show that Bellechasse soil has a much higher sorption capacity for AT than Moehlin soil. According to Domsch et al. (1992) the high sorption capacity of soils with high organic matter contents interferes with biodegradation. This may explain the low AT degrading activity of strain YAYA6 in Bellechasse soil. For the same reason, inoculation of compost with the mixed microbial community from which strain YAYA6 was isolated failed to accelerate AT biodegradation (data not shown).

When Moehlin soil was incubated for 3 weeks with 15 mg/kg AT and inoculated with strain YAYA6 after this preincubation period, the half-life of AT was found to be 2 times higher than in soil to which AT and the inoculum were added at the same time. This finding may be explained by a reduced bioavailability resulting from the strong binding of AT to soil components during the preincubation period (Domsch et al., 1992).

AT degradation was found to be slower in inoculated Schafisheim soil compared to Moehlin soil, despite comparable K_d values. This difference may be due to soil pH since from the work in liquid media it is known that the rate of AT degradation by strain YAYA6 is strongly reduced below pH 7 and above pH 9 (Yanze Kontchou and Gschwind, 1994). In uninoculated Schafisheim soil the half-life of AT was found to be 47 days compared to 55 days in Moehlin soil. Inoculation with YAYA6 reduced the half-life to 1 day in Moehlin soil and to 19 days in Schafisheim soil.

Survival of YAYA6 in Nonsterile Soils. Soils at 50% water content were inoculated with strain YAYA6 at a concentration to reduce AT half-life to 1 day in Moehlin soil. The inoculated soils were then incubated for 21 and 42 days at 22 °C in the dark. After this preincubation period, AT was added at a concentration of 10 mg/kg, and the residual AT concentration was measured 2 and 7 days after the addition of AT (Table 2). The estimated half-lives after 42 days of preincubation were 4–5 days in Moehlin soil, 25 days in Schafisheim soil, and over 50 days in Bellechasse soil. AT degradation in Moehlin soil after preincubation is much slower in the first 2 days after AT addition than between day 2 and day 7 (Table 2). This indicates that AT degradation starts after a lag period. AT degradation in soil after prolonged incubation of the bacteria in soil without the addition of a substrate is in contrast to the results of Goldstein et al. (1985) and Gunalan and Fournier (1993). Their bacterial strains enriched and isolated for the degradation of refractory xenobiotics did not survive and retain their activity in soil or surface water.

Table 2. Survival of YAYA6 in Nonsterile Soils

incubation period (days)		atrazine (%)		
before	after	Moehlin	Bellechasse	Shafisheim
21	2	87.1	96.6	79.5
	7	2.2	95.2	72.7
42	2	96.2	96.3	90.0
	7	8.8	94.4	82.2

[a] Different soils inoculated with strain YAYA6 were incubated for 21 and 42 days before the addition of AT at a concentration of 10 mg/kg; 2 and 7 days after the addition of AT, the remaining AT concentration was determined. The data shown are mean values from triplicate samples which differed by less than 5% in all cases.

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