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Arthrobacter sp. Strain KU001 Isolated from a Thai Soil Degrades Atrazine in the Presence of Inorganic Nitrogen Sources

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An atrazine-degrading bacterium, strain KU001, was obtained from a sugarcane field at the Cane and Sugar Research and Development Center at the Kasetsart University, Kamphaeng Saen Campus, Thailand. Strain KU001 had a rod-to-coccus morphological cycle during growth. Biolog carbon source analysis indicated that the isolated bacterium was *Arthrobacter histidinolovorans*. Sequence analysis of the PCR product indicated that the 16S rRNA gene in strain KU001 was 99% identical to the same region in *Arthrobacter* sp. The atrazine degradation pathway in strain KU001 consisted of the catabolic genes *trz*N, *atz*B, and *atz*C. Strain KU001 was able to use atrazine as a sole nitrogen source for growth, and surprisingly, atrazine degradation was not inhibited in cells grown on ammonium, nitrate, or urea, as compared with cells cultivated on growth-limiting nitrogen sources. During the atrazine degradation process, the supplementation of nitrate completely inhibited atrazine degradation activity in strain KU001, whereas ammonium and urea had no effect on atrazine degradation activity. The addition of strain KU001 to sterile or nonsterile soils resulted in the disappearance of atrazine at a rate that was 4- to 5-fold more than that achieved by the indigenous microbial community. The addition of citrate to soils resulted in enhanced atrazine degradation, where 80% of atrazine disappeared within one day following nutrient supplementation.

Keywords: Atrazine-degrading bacteria, *Arthrobacter*, inorganic nitrogen

The microbial degradation of atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-*s*-triazine) has been of interest since this chemical was first released into the environment about 50 years ago. Atrazine, which is one of the most widely used herbicides for the control of broad leaf weeds in corn, sorghum, and sugarcane [2], has been detected in surface and ground waters in the U.S. and other countries [2, 29]. Over the last 15 years, several laboratories have used enrichment culture strategies to independently isolate different Gram-negative bacteria capable of dechlorinating and, in some instances, mineralizing atrazine [3, 4, 14, 18, 19, 28, 33].

The Gram-negative bacterium *Pseudomonas* sp. strain ADP has been used as a model organism to dissect the enzymatic basis of atrazine mineralization [3, 7, 9, 10, 15, 20, 23, 25, 32]. In this bacterium, complete atrazine catabolism is initiated *via* three enzymatic steps encoded by the *atz*A, *atz*B, and *atz*C genes. The first enzyme, AtzA, catalyzes the hydrolytic dechlorination of atrazine to yield hydroxyatrazine, which is subsequently deamidated by AtzB to yield *N*-isopropylammelide. AtzC hydrolytically deaminates *N*-isopropylammelide, stoichiometrically, to cyanuric acid and *N*-isopropylamine. Cyanuric acid is subsequently hydrolyzed to carbon dioxide and ammonia by the combined activities of AtzDEF, which exist in an operon [15].

Other atrazine-degrading bacteria, including *Agrobacterium*, *Clavibacter*, *Rhizobium*, *Pseudomonas*, *Alcaligenes*, and *Ralstonia* strains, were previously shown to contain atrazine catabolic genes that are nearly identical to those found in *Pseudomonas* strain ADP [8, 32]. This suggested that horizontal gene transfer/transposition is involved in the dissemination of these genes amongst phylogenetically diverse Gram-negative soil bacteria. More recently, however, the Gram-positive bacteria *Nocardioides* sp. strains C190, SP12, and C157, *Arthrobacter aurescens* TC1, *Arthrobacter crystallopoietes*, and *Arthobacter* sp. strain AD1 have also been shown to use atrazine as sole nitrogen and, in some instances, sole carbon sources for growth [5, 17, 19, 27,

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30]. Interestingly, *Nocardioides* sp. strain C190 [30] has been shown to use atrazine as a sole source of N for growth, but does not contain the *atz*A, *atz*B, or *atz*C genes. In this bacterium, atrazine degradation is initiated by the product of the *trz*N gene, which has been cloned and sequenced [16] and expressed in *E. coli* [26]. Moreover, Piutti *et al.* [17] used PCR analyses to show that *Nocardioides* sp. strain SP12 contains a novel atrazine catabolic pathway combining *trz*N with *atz*B and *atz*C. Like *A. aurescens* strain TC1, this bacterium catabolizes atrazine to cyanuric acid *via* three enzymatic steps encoded by the hybrid pathway involving the *trz*N, *atz*B, and *atz*C genes [21]. More recently, many Gram-positive atrazine-degrading bacteria were isolated and shown to contain *trz*N and *atz*C [22, 31]. It seems that Gram-positive atrazine degraders are more likely to contain the *trz*N gene than the *atz*A gene. However, a Gram-positive *Arthrobacter* sp. has been reported to also contain *atz*A [5]. This combination of genes has been observed in other *Arthrobacter* and *Nocardioides* spp. strains [31]. Some of these bacteria had been investigated for atrazine degradation in the presence of an exogenous nitrogen source. It has been shown that some exogenous nitrogen-containing compounds inhibited the atrazine degradation activity [11, 12]. Additionally, some studies have shown that single bacterial strains or consortia are able to degrade atrazine in soil, but these organisms vary in their effectiveness.

In this study, an atrazine-degrading bacterium was isolated from an agricultural soil with a long history of atrazine applications in Thailand. The isolation and characterization of *Arthrobacter* sp. strain KU001 are described. The strain KU001 was capable of utilizing atrazine as a sole nitrogen source for growth. Additionally, the genes involved in the atrazine degradation pathway were determined by using the PCR technique. Furthermore, we investigated the influence of exogenous nitrogen sources on the atrazine degradation ability of strain KU001 in culture and the biodegradation of atrazine in soil microcosms.

MATERIALS AND METHODS

Isolation and Purification

Strain KU001 was isolated from soil samples obtained from a sugarcane field in the Cane and Sugarcane Research and Development Center at the Kasetsart University, Kamphaeng Saen Campus, Thailand. The site was chosen based on its history of atrazine applications (more than 10 years). A pure culture of strain KU001 was obtained by using the enrichment culture technique and dilution-plating and streaking on modified minimal R-medium [24] containing 1% glucose as carbon source and 500 μg/ml atrazine as nitrogen source (RGA medium).

Identification of Strain KU001

Microscopic determination. A pure culture of strain KU001 was grown in liquid R-medium, supplemented with 100 μg/ml atrazine as nitrogen source, for 18 and 33 h. One drop of culture was subjected to a Gram stain and placed on a glass microscope slide for cell morphology determination by using bright field illumination and an Olympus optical microscope equipped with a 100× objective.

Identification of strain KU001 using the Biolog Microlog system. The isolate was prepared according to Biolog instructions (Biolog, Inc., Hayward, CA, U.S.A.) that included culturing on Biolog Universal Growth (BUG) agar, preparation of a standardized liquid suspension based on turbidity, and inoculation of one GP2 MicroPlate per culture. After incubation at 37°C for 24 h, the MicroPlates were read on a Biolog plate reader and identified to species using the MicroLog System version 4.20 software. Standardized strains from ATCC for *E. faecalis, E. gallinarum,* and *E. faecium* were evaluated using the Biolog system and identified as such for quality assurance and quality control purposes.

16S rRNA gene sequence determination. A single-colony isolate was streaked onto RGA medium, picked using sterile loops, and suspended in 50 μl of sterile H_2O. One μl of the standardized cell suspension served as template DNA. The universal primers Un16S341f 5'-CCT ACG GGA GGC AGC AG-3' and Un16S1512r 5'-ACG GCT ACC TTG TTA CGA CTT-3' were used to amplify the 16S rRNA gene from strain KU001. Amplification was performed in a PTC-100 thermal cycler (MJ Research, Incline Village, NV, U.S.A.) using the following conditions: 5 min at 98°C, followed by 35 cycles of 30 s at 95°C, 1 min at 58°C, and 1 min at 72°C, with a final extension period of 2 min at 72°C. PCR fragments were amplified using *Taq* DNA polymerase (Fermentas, Canada) and detected on a 1.0% argarose gel. For direct sequencing, PCR products were purified and sequenced by Macrogen (Seoul, Korea).

Detection of Atrazine Degradation Gene Homologs in Strain KU001 by PCR Analysis

A single-colony isolate was streaked onto RGA medium, picked using sterile loops, and suspended in 50 μl of sterile H_2O. One μl of the standardized cell suspension served as template DNA, and the PCR technique was used to amplify sequences internal of the *atz*A, *atz*B, *atz*C, and *trz*N genes as previously described [8, 16]. The *atz*A, *atz*B, and *atz*C genes were amplified using the primers described by de Souza *et al.* [8], except that the primers for *atz*C were 5'-AGT CAG CGA AGG GCG TAG GTA TCA-3' and 5'-GAC AAA TCC GGG AGA CAC AAG GTT-3'. Primers for the amplification of *trz*N were used as described by Mulbry *et al.* [16]. PCR fragments were amplified using *Taq* DNA polymerase (Fermentas, Canada) and detected on a 1.0% agarose gel. For direct sequencing, PCR products were purified with the QIAquick PCR Purification Kit (Qiagen, Chatsworth, CA, U.S.A.) according to the manufacturer's directions. DNA concentrations of the purified PCR products were determined spectrophotometrically at 260 nm. DNA sequencing was performed by Macrogen (Seoul, Korea).

Use of Atrazine as Carbon or Nitrogen Sources

Bacterial growth studies were carried out in triplicate in 125-ml flasks, each containing 50 ml of R-medium supplemented with 100 μg/ml atrazine as the sole carbon or nitrogen sources. The cultures were incubated at 30°C on a rotary shaker at 150 rpm. Bacterial growth was measured by monitoring optical density at 600 nm (OD_{600}) every 3 h using a Beckman DU550 spectrophotometer (Beckman Coulter, Inc., CA, U.S.A.).

Effect of Growth on Different Nitrogen Sources on Atrazine Degradation

Arthrobacter sp. strain KU001 was grown in R-minimal medium containing 1% glucose and 0.12% ammonium chloride. The culture was incubated for 20 h at 30°C in a rotary shaker at 150 rpm. The cells were harvested at 8,000 ×*g* and washed three times with 0.85% NaCl and resuspended in R-minimal medium with ammonium sulfate ($(NH_4)_2SO_4$), sodium nitrate ($NaNO_3$), urea, or atrazine as the nitrogen source. In some experiments, atrazine was added as a second nitrogen source. Cultures were subsequently shaken at 30°C for 20 h and grown to the early exponential phase. Cells were harvested by centrifugation, and pellets were washed three times with buffer [10 mM sodium phosphate (pH 7), 0.1 mM $MgSO_4$] and resuspended in the same buffer to an OD_{600} of 0.3. Thirty ml of cell suspension was placed in flasks in a 30°C water bath and incubated for 2 min. Atrazine was then added to a final concentration of 15 μg/ml. Samples (1 ml) were withdrawn at 5-min intervals, centrifuged immediately for 3 min in a microcentrifuge at maximum speed, and the atrazine concentration in the samples was measured based on absorbance of the supernatants at 220 nm using a Beckman DU550 spectrophotometer (Beckman Coulter, Inc., CA, U.S.A.).

Effect of Inorganic Nitrogen Addition on Atrazine Degradation

This study additionally tested the effect of inorganic nitrogen source on the atrazine degradation activity of strain KU001. Strain KU001 was grown in 50 ml of R-minimal medium by shaking overnight at 30°C, with atrazine as the sole nitrogen source. Cells were harvested by centrifugation, washed three times with 0.85% NaCl, and resuspended in R-minimal medium to an OD_{600} of 0.3. Aliquots (30 ml) were placed in a water bath at 30°C and incubated for 2 min, and then atrazine was added to a final concentration of 15 μg/ml. In some experiments, $(NH_4)_2SO_4$, $NaNO_3$, or urea was added as a second nitrogen source. Samples (1 ml) were withdrawn at 5-min intervals, centrifuged immediately for 3 min in a microcentrifuge at maximum speed, and the atrazine concentration in supernatants was determined by measuring absorbance at 220 nm.

Atrazine-Degrading Efficacy of Stain KU001 in Soil Microcosms

Inocula for all microcosm studies were prepared by growing bacteria in 100 ml of R-medium supplemented with 100 μg/ml atrazine as nitrogen sources for 24 h at 30°C on a rotary shaker at 150 rpm. Cultures were pelleted by centrifugation at 8,000 ×*g* for 10 min at room temperature, cells were rinsed twice with 20 ml of sterilized 0.85% NaCl, and cell numbers were quantified by using the plate count techniques. Soil samples that had not been previously exposed to atrazine were collected for these experiments. The soil had the following characteristics: a sandy loam texture with 69% sand, 20% silt, and 11% clay; 0.73% organic C and 0.06% total N; and a pH of 7.63 (1:1 slurry). Soils were passed through a 2-mm sieve, and 150-g (dry weight basis) portions of nonsterilized and sterilized soils were adjusted to 8% moisture in the jam jars, and this moisture was maintained during the experiments. Soils were sterilized by autoclaving. Replicate soil microcosms were treated as follows: microcosm no. 1 was unamended and not inoculated; microcosm no. 2 was amended with 150 μg atrazine/g soil and not inoculated with KU001; microcosm no. 3 was amended with 150 μg atrazine/g soil and inoculated with KU001; microcosm no. 4 was amended with 150 μg atrazine/g soil and 1% citrate and inoculated with KU001; and microcosm no. 5 was the same as microcosm no. 3 except using sterilized soil. All soil microcosms were incubated at room temperature. Survival of strain KU001 was examined at 0, 1, 2, and 3 weeks after initiation of the studies and the atrazine concentrations were examined after 0, 1, 3, 5, and 7 days. Aliquots (10 g) of soil were removed from each container and extracted overnight with 30 ml of 1:1 (v/v) ethyl-acetate-hexane [14]. The extract was analyzed by gas chromatography as described below. The number of strain KU001 was determined by the plate count method.

Analytical Methods

Gas chromatography was performed by using a Shimadzu gas chromatograph model GC-2010 (Kyoto, Japan) equipped with an ECD detector. The fused silica capillary column Rtx -5 (30 m×0.25 mm, 0.25 μm) (Restek, Bellefonte, PA, U.S.A.) contained 5% diphenyl–95% dimethyl polysiloxane. Helium (purity >99.5%) was used as the carrier at 1 ml/min and nitrogen (99.9% purity) was used as makeup gas at 30 ml/min according to the optimization results of the instrument given by the manufacturer. The operating conditions were as follows: injector temperature, 240°C; detector temperature, 300°C. The column temperature program was 100°C, held for 1 min, and raised to 210°C (5°C/min) and held for 7 min.

RESULTS

Isolation and Identification of Strain KU001

A pure culture of an atrazine-degrading bacterium was isolated from sugarcane field soil by using the enrichment culture technique and restreaking on RGA medium. This pure culture was subsequently designated as strain KU001. Physiological characterization showed that the strain KU001 grew best at 25–30°C and colonies were circular and exhibited a yellow pigmentation on RGA medium. The maximal growth rate of the strain KU001 in minimal R-medium was 0.04 unit/h (Fig. 1). The microscopic morphology of strain KU001 was that of a rod shape during the exponential phase, and cells often appeared as V-shaped. At the stationary phase, however, strain KU001 cells

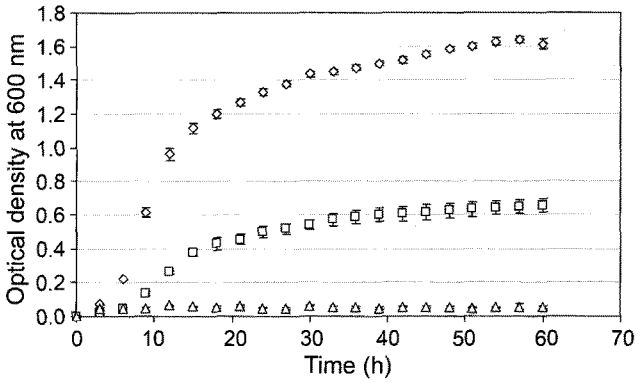

Fig. 1. Growth of the strain KU001 cultures in different media.
Symbols: ◇, R-medium; □, R-medium without NH_4Cl and supplemented with 100 μg/ml atrazine; △, R-medium without glucose and supplemented with 100 μg/ml atrazine (off scale).

appeared as short rods and some had cocci or coccoid-like morphologies. This is consistent with members of genus *Arthrobacter*. DePrada *et al.* [6] and Loveland *et al.* [13] described that all species in the *Arthrobacter* genus are rods during exponential growth and cocci in their stationary phase. Analysis of the test indicated that the strain KU001 was an *Arthrobacter histidinolovorans*, with Biolog similarity value of 100%. Analysis of the 16S rRNA gene sequence revealed that the sequence was most similar (99 % sequence identity) to that of the *Arthrobacter* sp. 16S rRNA gene. The nucleotide sequences of the 16S rRNA gene from strain KU001 was deposited in GenBank as Accession No. GU121476. Therefore, this bacterium was named *Arthrobacter* sp. strain KU001.

Detection of Atrazine Degradation Gene Homologs in Strain KU001 by PCR Analysis

To determine if *Arthrobacter* sp. strain KU001 contained *s*-triazine degradation genes homologous to those in *Pseudomonas* ADP, *Nocardioides* sp. strain C190, or *A. aurescens* strain TC1, we attempted to amplify *trz*N, *atz*A, *atz*B, and *atz*C genes using standard canonical primers. Results showed amplification of 0.4-kb *trz*N, 0.5-kb *atz*B, and 0.4-kb *atz*C fragments using strain KU001 as template DNA (data not shown). The *atz*A gene was not detected in this bacterium. Based on these results, strain KU001 likely degrades atrazine through hydroxyatrazine, *N*-isopropylammelide, and cyanuric acid. Like *A. aurescens* strain TC1, this bacterium catabolizes atrazine to cyanuric acid *via* three enzymatic steps encoded by the hybrid pathway involving the *trz*N, *atz*B, and *atz*C genes. Sequence analysis of the PCR products indicated the TrzN, AtzB, and AtzC homologs in *Arthrobacter* sp. strain KU001 were 100%, 99%, and 100% identical to the same region in *A. aurescens* strain TC1 (data not shown) [21]. This suggested that TrzN most likely initiates atrazine dechlorination in *Arthrobacter* sp. strain KU001.

Use of Atrazine as Carbon and Nitrogen Sources

When strain KU001 was grown in R-medium containing 1% glucose as carbon source and 100 μg/ml atrazine as the sole nitrogen source, cell densities reached OD_{600} of 0.6. The maximal growth rate of the strain KU001 in R-medium supplanted with ammonium or 100 μg/ml atrazine as the sole nitrogen source was 0.04 unit/h and 0.013 unit/h, respectively. However, when the strain KU001 was grown in R-medium supplemented with 100 μg/ml atrazine as carbon source, no growth was detected (Fig. 1). The Gram-positive bacteria *Nocardioides* sp. strains C190, SP12, and C157, *Arthrobacter aurescens*, *Arthrobacter crystallopoietes*, *Arthobacter* sp. strain AD1, as well as *Arthrobacter* sp. strain KU001 have been shown to use atrazine as sole nitrogen and, in some instances, sole carbon sources for growth [5, 17, 19, 27, 30].

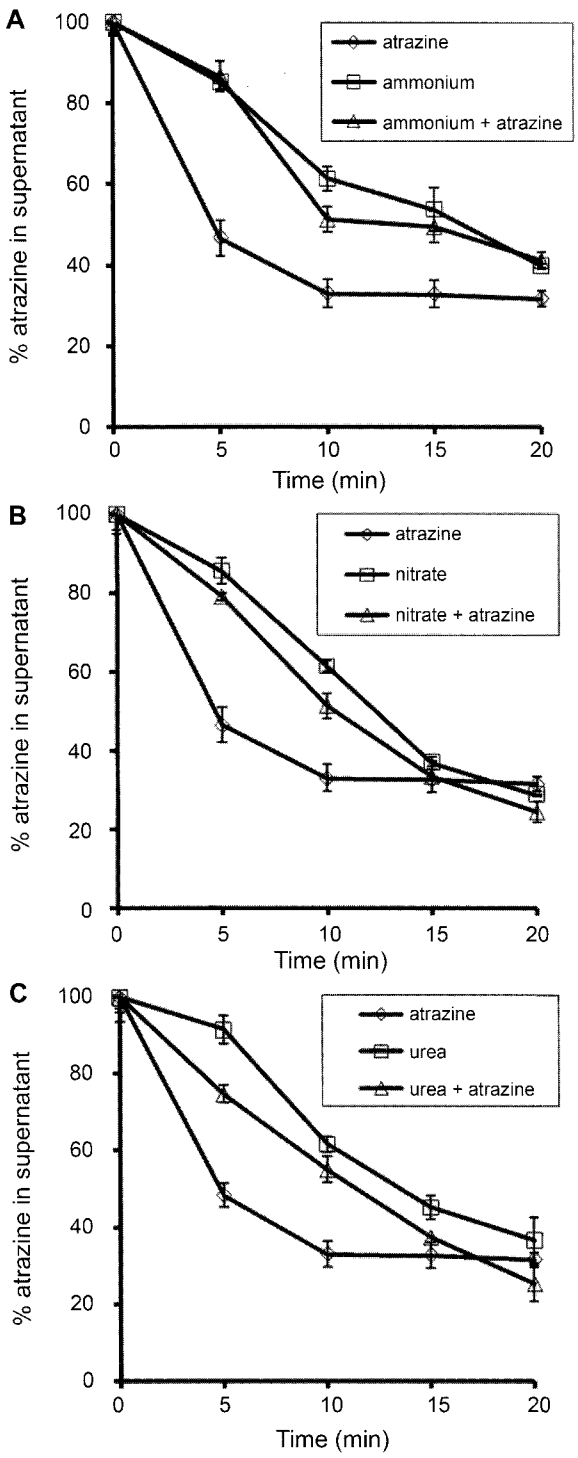

Fig. 2. Atrazine degradation in resting cells of the strain KU001 grown on different nitrogen sources.

The percentage of the initial atrazine concentration remaining in the supernatant at each time point is plotted against time. Each panel represents the results obtained with different nitrogen sources. **A**. Ammonium sulfate; **B**. Sodium nitrate; **C**. Urea. The plot obtained with atrazine-grown cells is displayed in all panels for reference. Symbols: ◇, cells grown on atrazine as the sole nitrogen source; □, cells grown on each compound as the sole nitrogen source; △, cells grown on each compound plus atrazine as nitrogen sources.

The Ability to Degrade Atrazine in The Presence of Inorganic Nitrogen Sources

A resting cell assay was used to examine atrazine degradation when cells were grown on different nitrogen sources. Cells grown on $(NH_4)_2SO_4$, $NaNO_3$, or urea as the sole nitrogen source did not inhibit the ability of strain KU001 to degrade atrazine in the supernatant (Fig. 2A to 2C). Atrazine degradation occurred almost at the same rate after cultivation on growth-limiting nitrogen sources medium in the presence of atrazine, ammonium, nitrate, or urea. However, atrazine degradation in cells grown in medium containing only atrazine as the sole nitrogen source showed the highest rate of atrazine degradation within 10 min. Unexpectedly, the atrazine degradation ability of strain KU001 was maintained during cultivation on growth medium containing inorganic nitrogen. Strain KU001 grown on medium containing atrazine as the sole nitrogen source degraded approximately 70% of atrazine within 10 min, the same rate as seen when strain KU001 was grown on medium containing atrazine in addition to the other tested nitrogen sources.

To determine if the nitrogen control of atrazine degradation may cause a constraint to degradation of atrazine by strain KU001 in nitrogen-rich agricultural soils, we added inorganic nitrogen sources to R-medium containing KU001 and atrazine. Results of these studies revealed that the atrazine degradative activity in strain KU001 can be completely inhibited in the presence of nitrate as the exogenous inorganic nitrogen source (Fig. 3). In contrast, the presence of ammonium or urea had no effect on atrazine degradation, either as an inhibitor or stimulator. Similar results were found in the soil microcosm experiments (data not shown).

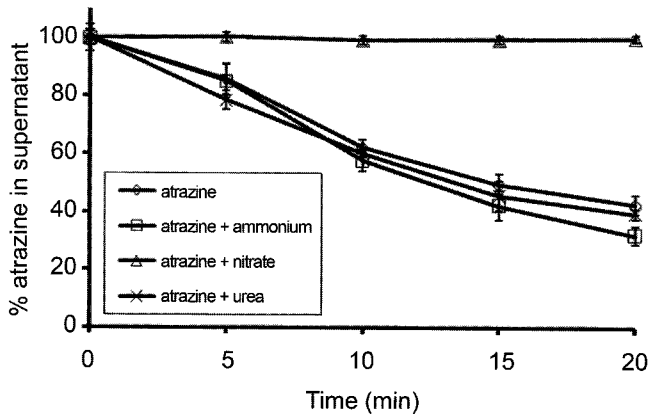

Fig. 3. Effect of exogenous nitrogen sources on the atrazine degradation activity of strain KU001 during the degradation process.

The percentage of atrazine concentration remaining in the supernatant at each time point is plotted against time. Symbols: ◇, cells in R-medium supplemented with atrazine; □, cells in R-medium supplemented with atrazine plus ammonium sulfate; △, cells in R-medium supplemented with atrazine plus sodium nitrate; ×, cells in R-medium supplemented with atrazine plus urea.

Atrazine-Degrading Efficacy of Stain KU001 in Soil Microcosm

The addition of strain KU001 to sterile or nonsterile soil resulted in approximately 100% disappearance of 150 μg atrazine/g soil within 5 days (Fig. 4). The soil without added strain KU001 degraded approximately 25% of the atrazine within 5 days, indicating the presence of indigenous, atrazine-degrading bacteria. Additionally, soils treated with 150 μg atrazine/g soil, and inoculated with strain KU001 and supplemented with 1% citrate, transformed 80% of the added atrazine within one day, compared with soil inoculated with strain KU001 but without citrate. This indicated that citrate addition stimulated the atrazine disappearance. However, the results of these related experiments revealed that the addition of only citrate without strain KU001 did not stimulate the atrazine disappearance (data not shown). Cell viability assays done using plate counts showed a loss of cell viability after 3 weeks, with cell numbers of 7×10^7, 5×10^7, 2.5×10^7, and 1×10^7 CFU/g soil at 0, 1, 2, and 3 weeks, respectively, in all treatments.

Discussion

We isolated an atrazine-degrading bacterium, *Arthrobacter* sp. strain KU001, from a Thai agricultural soil using the enrichment culture technique. This atrazine-degrading isolate was obtained after 1 week of incubation of soil in minimal R-medium supplemented with 100 μg/ml atrazine as the sole nitrogen source. Several other laboratories have

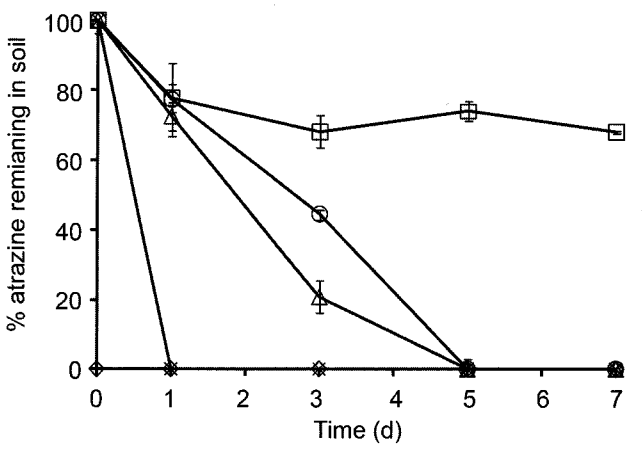

Fig. 4. Atrazine degradation of 150 μg atrazine/g soil added to soil.

Nonsterile soils were amended with strain KU001 (1×10^8 cell/g soil) or both strain KU001 and 1% citrate, and sterile soil were amended with strain KU001 for 7 days after atrazine treatment. Symbols: ◇, nonsterile soil without atrazine (off scale); □, nonsterile soil amended with atrazine; △, nonsterile soil amended with atrazine and inoculated with strain KU001; ×, nonsterile soil amended with atrazine and inoculated with strain KU001 with citrate added; ○, sterile soil amended with atrazine and inoculated with strain KU001.

independently isolated Gram-positive bacteria in the genera *Nocardioides* and *Arthrobacter* that use atrazine as the sole nitrogen and, in some instances, sole carbon sources for growth [5, 17, 19, 27, 30]. Although some of these microorganisms have the *atz*A, *atz*B, and *atz*C genes, others do not [5, 19, 27, 30]. Bacteria such as *Nocardioides* sp. strains C190 and SP12 and *A. aurescens* strain TC1 initiate atrazine catabolism *via* the activity of TrzN, which has been sequenced [16, 17, 21, 30], whereas *Arthrobacter* sp. AD1 uses AtzA to dechlorinate atrazine [5]. Presently, several different ways of atrazine degradation have been reported in bacteria. Bacteria such as *Pseudomonas* ADP generally initiate atrazine catabolism using AtzA, AtzB, and AtzC [32], whereas others like *Arthrobacter* sp. strain KU001 contain a hybrid atrazine catabolic pathway combining *trz*N with *atz*B and *atz*C. These results indicate that nearly identical *trz*N genes are found among members of the genera *Nocardioides* and *Arthrobacter* [16, 17, 21, 30].

Our data clearly show that the addition of ammonium, nitrate, or urea in the growth medium had no inhibitory effect on the catabolic pathway. In contrast, in *Pseudomonas* strain ADP and isolate M91-3, the presence of exogenous inorganic nitrogen during the degradation process inhibited subsequent atrazine degradation activity in washed-cell suspensions [12, 13]. This suggests that the regulation of atrazine and nitrogen metabolism may not be related in strain KU001 and previously identified bacteria. However, the atrazine degradation rate was decreased in cells grown on nitrogen sources that support growth of *Arthrobacter* sp. strain KU001 compared with cells cultivated on growth-limiting nitrogen sources. Atrazine degradation in cells grown in medium containing atrazine as the sole nitrogen source showed the highest rate of atrazine degradation. Moreover, in this study, we investigated the effect of inorganic nitrogen addition on the atrazine degradative activity in an active atrazine-degrading strain, KU001, during the degradation process. We found that the presence of inorganic nitrogen sources, in the form of ammonium or urea, was not detrimental to atrazine degradation by strain KU001. On the other hand, nitrate addition inhibited atrazine degradation by strain KU001. This indicates that strain KU001 is able to degrade atrazine in atrazine-contaminated agricultural soils amended with ammonium and urea fertilizer. These results suggested that strain KU001 can be used for the bioremediation of atrazine-contaminated sites by the presence of inorganic nitrogen fertilizers in the form of ammonia and urea. Taken together, these findings have significant implications for the environmental fate of atrazine in agricultural land since these herbicides are frequently applied to soils receiving N fertilizers.

The addition of strain KU001 to sterile or nonsterile soils resulted in 4 to 5-folds more atrazine disappearance than that by the indigenous microbial community. Many studies have shown that single bacterial strains or consortia are able to degrade atrazine in soil, but these organisms vary in their effectiveness. *Pseudomonas* strain ADP was shown to degrade 17% of 1,500 μg atrazine/g soil, but degradation in soil increased up to 70% with the addition of citrate [14]. Another *Pseudomonas* strain YAYA6 mineralized over 60% of 10 μg atrazine/g soil within 49 days [33, 34]. The strain YAYA6 degraded atrazine much more slowly in a soil with low pH and high organic matter content. Assaf and Turco [1] isolated mixed bacterial cultures that had the ability to transform 87% of 30.2 μg atrazine/g soil within 25 days and to convert 60% of the applied atrazine to CO_2 within 30 days after inoculation. The addition of *Agrobacterium radiobacter* J14a into soil with a low indigenous population of atrazine degraders treated with 50 and 200 μg atrazine/g soil resulted in two to five times higher mineralization than that found in the noninoculated soil [28]. The addition of sucrose to inoculated strain J14a soil had no effect on mineralization, whereas the addition of citrate to inoculated strain ADP and KU001 resulted in stimulation of atrazine disappearance. Altogether, these studies indicate a broad variation in the kinetics and extent of atrazine degradation and the ability of bacteria to degrade atrazine. In addition, the stimulatory or inhibitory effects of additional carbon or nitrogen sources on atrazine degradation vary among the bacteria described in the literature. An understanding of these factors is necessary in order to improve the capability of these bacteria in biodegradation and bioremediation applications.

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