Biodegradation (2010) 21:97–105
DOI 10.1007/s10532-009-9284-9

ORIGINAL PAPER

Atrazine degradation by a simple consortium of *Klebsiella* sp. A1 and *Comamonas* sp. A2 in nitrogen enriched medium

Chunyu Yang · Yang Li · Kun Zhang · Xia Wang · Cuiqing Ma · Hongzhi Tang · Ping Xu

Received: 22 February 2009 / Accepted: 1 July 2009 / Published online: 15 July 2009

Abstract A simple consortium consisted of two members of *Klebsiella* sp. A1 and *Comamonas* sp. A2 was isolated from the sewage of a pesticide mill in China. One member of *Klebsiella* sp. A1 is a novel strain that could use atrazine as the sole carbon and nitrogen source. The consortium showed high atrazine-mineralizing efficiency and about 83.3% of 5 g l^{-1} atrazine could be mineralized after 24 h degradation. Contrary to many other reported microorganisms, the consortium was insensitive to some nitrogenous fertilizers commonly used, not only in presence of 200 mg l^{-1} atrazine but also in 5 g l^{-1} atrazine mediums. After 24 h incubation, 200 mg l^{-1} atrazine was completely mineralized despite of the presence of urea, $(NH_4)_2CO_3$ and $(NH_4)_2HPO_4$ in the medium. Very minor influence was observed when NH_4Cl was added as additional nitrogen source. Advantages of the simple consortium, high mineralizing efficiency and insensitivity to most of exogenous nitrogen sources, all suggested application potential of the consortium for the bioremediation of atrazine-contaminated soils and waters.

C. Yang · Y. Li · K. Zhang · X. Wang · C. Ma · P. Xu
State Key Laboratory of Microbial Technology, Shandong University, 250100 Jinan, People's Republic of China

H. Tang · P. Xu (✉)
MOE Key Laboratory of Microbial Metabolism, School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, 200240 Shanghai, People's Republic of China
e-mail: pingxu@sjtu.edu.cn

Keywords Atrazine · Bioremediation · Consortium · *Klebsiella* · Nitrogen insensitivity

Introduction

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine) is the herbicide that has been widely used in the world to control broadleaf weeds in the past 30 years. Due to its high mobility and long half life in soil, residues of both the parent compound and its derivatives have been detected in soil, surface, water and groundwater after year's application (Schiavon 1988). Most recently, atrazine present at ppb levels has shown to disrupt sexual development in amphibians and thus may pose serious ecological risks (Rhine et al. 2003). As a principal process of atrazine mineralization, bioremediation in atrazine contaminated soil and water is necessary and received many attentions around the world. Many microorganisms were isolated and studied for their abilities in atrazine mineralization including members of genera *Pseudomonas*, *Acinetobacter*, *Agrobacterium*, *Arthrobacter*, *Rastonia* and *Norcardioides* (Eaton and Karns 1991; Yanzekontchou and Gschwind 1994; Bouquard et al. 1997; Struthers et al. 1998; Strong et al. 2002). Of all the studied bacteria, *Pseudomonas* sp. strain ADP might be the best-characterized atrazine mineralizing one (Mandelbaum et al. 1995). The genes encoding the three enzymes which are responsible for the conversion of atrazine to cyanuric acid were *atzA, B, C*,

respectively. They are found on a self-transmissible plasmid and thought to be highly conserved in many isolated bacteria (de Souza et al. 1998b; de Souza et al. 1998c).

Different from the gram-negative isolate of *Pseudomonas* sp. ADP, gram-positive isolate of *Norcardioides* sp. C190 was reported to contain *trzN* gene and another unknown gene, which encoded the chlorohydrolase enzyme and the enzyme catalyzing the reaction of deisopropylammelide, respectively (Topp et al. 2000). In a recent report, *Nocardioides* sp. SP12 was found to combine the genes of *trzN* with *atzB* and *atzC*, which encoded the enzymes metabolizing atrazine to cyanuric acid (Piutti et al. 2003).

In the natural environment, organic compound degradation is often carried out by a mixed microbial community. Evidences suggested that rates of growth and substrate utilization are frequently higher in enriched mixed cultures than those rates in pure cultures isolated from the mixture (Weightman and Slater 1988). For atrazine degradation, bacteria consortia appeared to be more common and more efficient than individual species (Mandelbaum et al. 1993; Assaf and Turco 1994), and some consortia were reported for their metabolic cooperative actions by investigating the individual's contribution in atrazine degradation (de Souza et al. 1998a; Smith et al. 2005).

Agricultural soils are often rich in nitrogen due to routine fertilization. It is well known that most atrazine-degrading bacteria use atrazine as a nitrogen source (Alvey and Crowley 1995; Abdelhafid et al. 2000b; Garcia-Gonzalez et al. 2003). The influence of nitrogen compounds on the efficiency of atrazine catabolism would be a major drawback in atrazine biodegradation. Therefore, seeking for non-responsive microorganisms to exogenous nitrogen sources is necessary for practical bioremediation. But to our knowledge, only a few reports have been emphasized on the exogenous nitrogen influence on atrazine degradation (Entry et al. 1993; Radosevich et al. 1995; Gebendinger and Radosevich 1999; Abdelhafid et al. 2000a), and most microorganisms were found sensitive to exogenous nitrogen source in atrazine degradation.

In present study, a simple consortium which consisted of two strains of *Klebsiella* sp. and *Comamonas* sp. was isolated for its high capacity in atrazine mineralization. The bioremediation potential of the consortium was investigated by its insensitivity to some exogenous nitrogen sources.

Materials and methods

Chemicals

Atrazine (97% purity) was gift from Sheng-bang Lv Ye Pesticide Corporation in China. Cyanuric acid (1,3,5-*s*-triazine-2,4,6-triol) and hydroxyatrazine (2-hydroxy-4-ethylamino-6-isopropylamino-1,3,5-*s*-triazine) were purchased from Sigma–Aldrich Chemical Co.

Isolation of microorganisms

The consortium was isolated from the sewage of a pesticide mill which has manufactured atrazine for about 8 years. Enrichment medium consisted of a mineral salts medium and 0.5 g l^{-1} atrazine as the sole carbon and nitrogen source, inoculated with soil (25%, w/v) and incubated aerobically with shaking at 150 rpm at 30°C. The mineral salts medium (MM) contained (per liter) 0.6 g of K_2HPO_4, 2.5 g of KH_2PO_4, 0.3 g of $MgSO_4{\cdot}7H_2O$ and 0.4 g of NaCl. The pH of the medium was adjusted to 7.0 with 4 M NaOH solution. Cultures were spreaded onto agar plates of MM with 0.5 g l^{-1} atrazine as the sole carbon and nitrogen source, big colonies were selected for the atrazine-degradation ability determination. Pure cultures were obtained by plating the enrichment culture onto MM agar amended with glucose or lactic acid as extra carbon sources.

16S rRNA gene sequence analysis

The genomic DNA of the strains was extracted and precipitated following the standard protocol for bacterial genomic DNA preparations (Sambrook et al. 1989). The partial 16S rRNA genes were amplified by polymerase chain reaction (PCR) using the universal primers of 16S rRNA gene. The oligonucleotide primers used were the forward primer (CCGGATC CAGAGTTTGATCCTGGCTCAG) and the reverse primer (CGGGATCCTACGGCTACCTTGTTACGA CT). Each PCR mixture contained 1.5 U of *Taq* polymerase, 5 µl 10 × *Taq* buffer, 0.2 mM concentration of each deoxynucleoside triphosphate, 0.6 µM primers and 0.5 µl of DNA template. The PCR cycle

parameters were as follows: 94°C for 4 min, 20 cycles of 94°C for 1 min, 50°C for 1.5 min and 72°C for 2 min. The DNA sequences were analyzed using the BLAST program of the National Centre for Biotechnology Information (NCBI).

The 95 carbon sources utilization by *Comamonas* sp. A2 was tested by Biolog Microbial Identification System (Biolog, Calif).

Estimation of bacterial atrazine-degrading capacity

Precultures of *Klebsiella* sp. A1 was incubated in MM medium with 0.2 g l^{-1} atrazine as nitrogen source and 1 g l^{-1} glucose as carbon source; strain of *Comamonas* sp. A2 was cultivated in MM with 1.5 g l^{-1} atrazine as nitrogen source and 1 g l^{-1} lactic acid as carbon source; the consortium was cultivated in MM amended with 1 g l^{-1} glucose and 1 g l^{-1} atrazine. The inoculums were washed three times and resuspended with MM to an optical density at 620 nm (OD_{620}) of 1.5, and then inoculated into MM by transferring 10% (v/v) culture volume. Degradation was conducted in 500 ml flasks containing 50 ml MM. The atrazine concentrations in the mixtures degraded by *Klebsiella* sp. A1, *Comamonas* sp. A2 and the consortium were 1 g l^{-1}, 8 g l^{-1} and 5 g l^{-1}, respectively. One gram per liter lactic acid was added to the degrading mixture of *Comamonas* sp. A2 as additional carbon source. For the preparation of cells and atrazine degradation, pure isolates and their combination were all cultivated at 30°C and aerated at 150 rpm. Atrazine was quantified by HPLC (Aglient 1100) equipped with a variable-wavelength UV detector and fitted with a reverse-phase C_{18} column (length 15 cm, internal diameter 4.6 mm, Hewlett-Packard). The solvent system consisted of methanol-water (70/30, v/v) delivered at a flow rate of 0.5 ml min^{-1}. The aqueous samples used for HPLC analysis were prepared by adding three times volume of methanol to samples, precipitated at 4°C for 4 h and then centrifuged at 11,160×*g* for 10 min to remove the precipitated debris.

Metabolites identification by resting cells

For metabolites identification, cells of the consortium were cultivated for 24 h and centrifuged at 6,869×*g* for 5 min to harvest cells and the pellets were washed three times with MM. The pellet was resuspended in MM at an optical density at 620 nm (OD_{620nm}) of 5.0 and amended with 10 g l^{-1} atrazine. The suspension with atrazine amendment was incubated at 30°C, sampled at 2-h intervals and analyzed by HPLC-Mass spectrometry (MS) with a MAT 2010 mass spectrometer (2010 EV, Shimadzu) equipment. The ESI temperature was 250°C and the turbo potential temperature was set at 400°C, with the declustering potential set at 60 V. The mobile phase and flow rate were the same as those described in HPLC analysis.

Atrazine-degrading genes analysis

Total genomic DNA was extracted as above described and used as the PCR template. The PCR primers were designed and synthesized as follows: *atzA, B, C* (de Souza et al. 1998c), *atzD* (Smith et al. 2005), *atzE, F* (Piutti et al., 2003) and *trzD* (Rousseaux et al., 2001), *N* (Mulbry et al. 2002). PCR reaction was performed with the following thermocycle program: 95°C for 5 min, 30 cycles of 92°C for 1 min, 55°C for 30 s, 72°C for 1 min, and a final extension for 6 min at 72°C. The PCR products were purified with a Qiagen II extraction kit (Qiagen Corp., Germany), ligated into pMD-18T vector (Promega) and followed by transformation into *E.coli* DH5α competent cells. Sequencing was conducted on an ABI 3700 capillary sequencer. The resulting sequences were compared with the genes available in the GenBank nucleotide library by a BLAST search through the National Center for Biotechnology Information (NCBI) Internet site (http://www.ncbi.nlm.nih.gov/BLAST/).

To locate the atrazine-degrading genes in *Klesiellleae* sp. A1, southern hybridization experiments were performed using a DIG DNA labeling and detection kit (Roche). Probes were prepared by random primer labeling with digoxigenin according to the manufacturer's instructions. Hybridization was performed overnight to detect the *atzA, B, C* genes in *Klebsiella* sp. A1. Filters (positively charged nylon transfer membranes) were washed under high-stringency conditions: twice for 5 min at room temperature in 2 × SSC-0.1% sodium dodecyl sulfate and then twice for 15 min at 68°C in 0.1 × SSC-0.1% sodium dodecyl sulfate (1 × SSC is 0.15 M NaCl plus 0.015 M sodium citrate). The 0.5 kb, 0.5 kb and 0.6 kb PCR fragments of *atzA, B, C* genes, obtained

from *Comamonas* sp. A2, were used as templates for preparing probes, respectively. The genomic DNA was digested with restriction enzymes of *Eco*RI and *Hin*dIII by standard procedures. The procedures used to prepare dot blots and southern blots were according to the methods described by Sambrook et al. (1989).

Effect of exogenous nitrogen on atrazine degradation

To investigate the effect of exogenous nitrogen sources on the activity of the consortium in atrazine degradation, MM with 200 mg l^{-1} or 5 g l^{-1} atrazine were prepared amended with exogenous nitrogen of NH_4Cl, NH_4NO_3, $(NH_4)_2HPO_4$, $(NH_4)_2SO_4$, urea and $(NH_4)_2CO_3$, respectively. The nitrogen content of these exogenous nitrogen sources in the mixtures were all 2 g l^{-1} and the pH of the mixtures was adjusted to 7.0 after each nitrogen source was added separately. Moreover, to further demonstrate the influence of exogenous nitrogen source, 200 mg l^{-1} atrazine was degraded in MM amended with 4.3 g l^{-1} urea (2 g l^{-1} nitrogen content) as exogenous nitrogen source or not. After inoculation, the concentrations of atrazine and bacterial growth were measured every 4-h incubation.

Nucleotide sequence accession number

The 16S rRNA gene sequences of *Klebsiella* sp. A1 and *Comamonas* sp. A2 are deposited in GenBank with the accession numbers of EU016084 and EU016085, respectively. The accession numbers of *atzA, B, C, D* in *Comamonas* sp. A2 are from GQ325256 to GQ325259.

Results

Isolation and identification of atrazine-degrading microorganisms

Occasionally, a consortium which initially grew together and appeared as a single colony was selected from the screening plate. After purification for three times, the consortium was isolated as two bacteria. 16S rRNA gene analysis indicated the strain A1 showed high similarity of 99.7% with *Klebsiella ornithinolytica* and strain A2 exhibited 99.3% similarity with *Comamonas testosterone*. Also Biolog determination confirmed that strain A2 had 99.0% similarity with *Comamonas testosterone*. *Comamonas testosterone* has very narrow-spectrum substrate profile (Bergey and Holt 1994). Biolog substrates utilization further confirmed that the strain A2 could only use DL-lactic acid, but not glucose and sucrose as carbon source.

Atrazine degradation by the consortium and pure cultures

When atrazine served as the sole carbon and nitrogen source, 83.3% of 5 g l^{-1} atrazine was mineralized after 24 h incubation by the consortium (Fig. 1c). To our knowledge, it is the highest degrading efficiency reported in atrazine degradation. However, Fig. 1a suggested that *Klebsiella* sp. A1 showed poorer growth as well as atrazine degrading abilities in MM medium, with only 45% of 1 g l^{-1} atrazine degraded by the isolate after 24 h incubation and 0.51 OD_{620nm} obtained (data not shown). No growth or atrazine degrading ability was detected by *Comamonas* sp. A2 when atrazine served as the sole carbon and nitrogen source. The strain could use atrazine as the sole nitrogen source. Additional carbon sources influence on atrazine degradation by *Comamonas* sp. A2 suggested that lactic acid addition could stimulate its growth and degradation ability to a large extent. After 24 h incubation higher efficiency of about 87% of 8 g l^{-1} atrazine mineralization was obtained (Fig. 1b). This coincided with the Biolog analysis that the strain has very narrow substrate-spectrum, lactic acid instead of glucose was one of the substrates that could be utilized by the bacterium (Bergey and Holt 1994).

Identification and biodegradation of the metabolites

When atrazine was mineralized by the consortium, three metabolites from atrazine was identified with the mass ion at m/z of 198.1 (M + 1) (Fig. 2a), 171.1 (M + 1) (Fig. 2b) and 129.9 (M + 1) (Fig. 2c) by MS analysis. With the reference information of standard compounds and reported atrazine degradation pathway, these compounds were proposed as hydroxyatrazine, N-isopropylammelide, and cyanuric

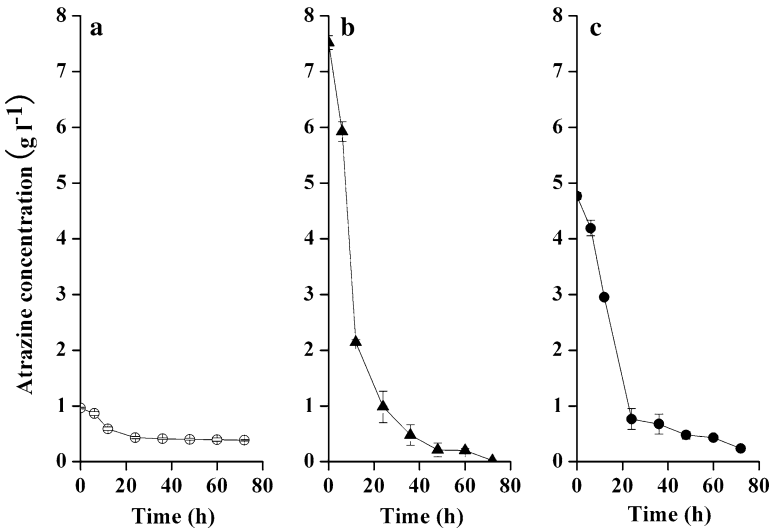

Fig. 1 Degradation curves of atrazine by the pure isolates or by the consortium. *Symbols*: for 1 g l^{-1} atrazine degraded by *Klebsiella* sp. A1 with atrazine as the sole carbon and nitrogen source (○); for 8 g l^{-1} atrazine degraded by *Comamonas* sp. A2 in lactic acid medium (▲); for 5 g l^{-1} atrazine degraded by the consortium with atrazine as the sole carbon and nitrogen source (●)

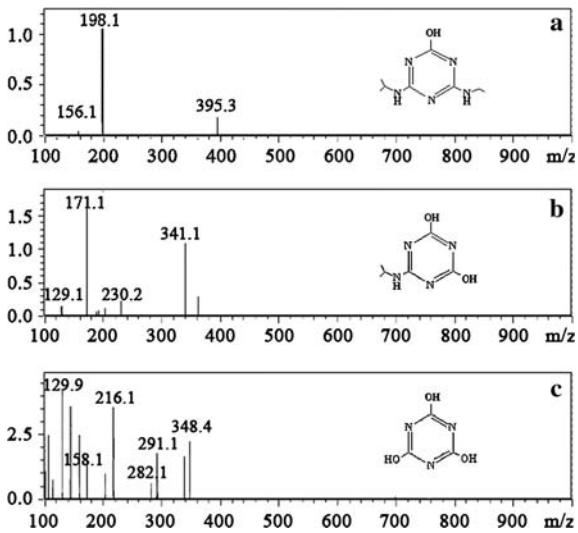

Fig. 2 HPLC-MS identification of the metabolites produced from atrazine by the consortium. **a** Represents hydroxyatrazine; **b** Represents N-isopropylammelide and **c** represents cyanuric acid

acid, respectively (De Souza et al. 1998b). HPLC spectrum suggested that no excessive metabolites accumulation during the 5 g l^{-1} atrazine-degrading process by the consortium (Fig. 3). The hydroxyatrazine reached its highest concentration of 35 mg l^{-1} after 6 h mineralization, and then showed obvious decreasing trend. At the meanwhile, cyanuric acid reached its peak concentration of 20 mgl^{-1} after 48 h mineralization, and then decreased quickly.

Atrazine-degrading genes in *Klebsiella* sp. A1 and *Comamonas* sp. A2

The expected 0.5 kb, 0.5 kb, 0.6 kb, 0.5 kb, 0.25 kb, and 0.25 kb PCR products of *atzA, B, C, D, E, F* genes were all obtained from *Comamonas* sp. A2. Sequences comparison revealed that the *atzA* gene contained in *Comamonas* sp. A2 shows 99.2% identity with the gene in *Pseudomona*s sp. ADP (U66917), *atzB, C, E, F* genes all exhibit 100% and *atzD* gene with 99.8% identities with those of *Pseudomonas* sp. ADP (U66917). However, although conducted at various PCR conditions, these primers failed to amplify genomic DNA of strain A1. Southern hybridization further indicated the absence of *atzA, B, C, D* genes in *Klebsiella* sp. A1. No products were obtained from both *Klebsiella* sp. A1 and *Comamonas* sp. A2 (data not shown) when using the primers of *trzD* and *trzN* genes for amplification.

Influence of nitrogen source on atrazine biodegradation

For their frequent employment in agriculture, six nitrogen sources were selected to investigate the effect of exogenous nitrogen on atrazine degradation. As shown in Fig. 4, compared with the degradation in which atrazine served as nitrogen source separately, regardless of the existence of urea, $(NH_4)_2CO_3$ and $(NH_4)_2HPO_4$ in the medium, 200 mg l^{-1} atrazine

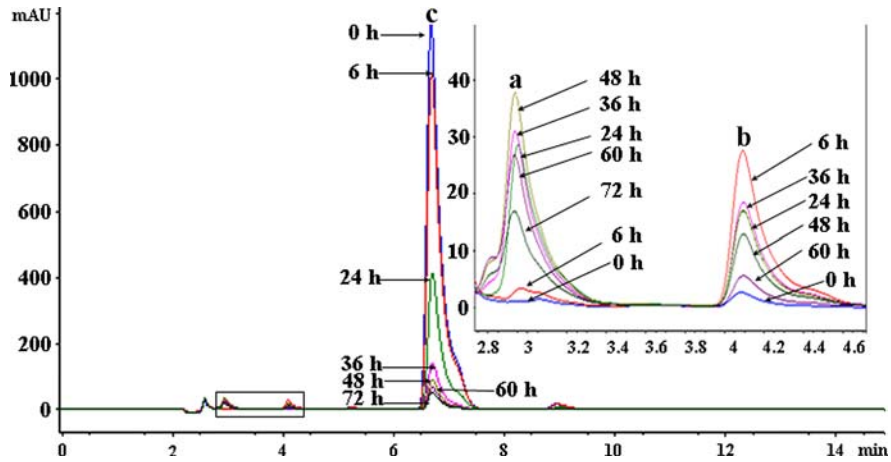

Fig. 3 HPLC analyses the variations of atrazine, hydroxyatrazine, and cyanuric acid during atrazine-degradation process by the consortium. **a** Represents cyanuric acid, **b** Represents hydroxyatrazine and **c** represents atrazine

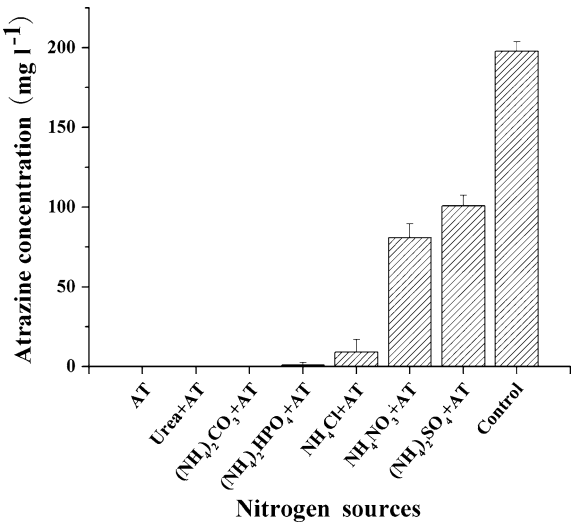

Fig. 4 Influence of additional nitrogen source on 200 mg l^{-1} atrazine degradation by the consortium

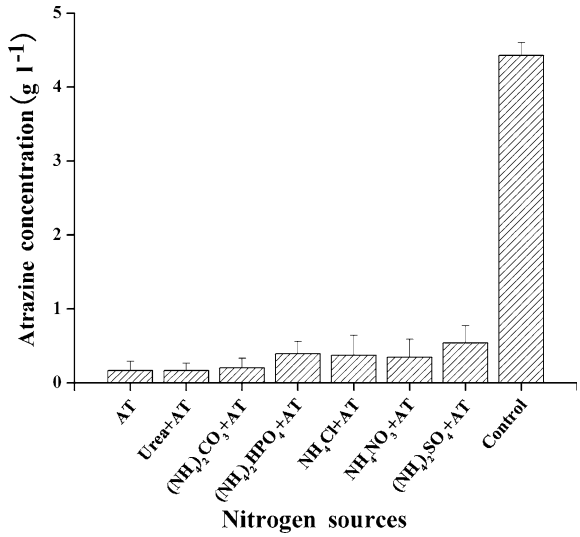

Fig. 5 Influence of additional nitrogen source on 5 g l^{-1} atrazine degradation by the consortium

(AT) was mineralized after 24 h incubation. This equivalent to the level of control, in which atrazine was degraded without any exogenous nitrogen added. Very minor influence was observed when NH_4Cl added as additional nitrogen source, in which atrazine decreased from 200 mg l^{-1} to 9.0 mg l^{-1}. Relatively, the addition of $(NH_4)_2SO_4$ and NH_4NO_3 resulted in lower atrazine mineralization, in which atrazine concentration decreased to 80.8 mg l^{-1} and 100.7 mg l^{-1}, respectively. As shown in Fig. 5, when high concentration of 5 g l^{-1} atrazine was degraded by the consortium in the presence of the selected six exogenous nitrogen sources, except in the mixture with $(NH_4)_2SO_4$ amendment (atrazine final concentration was 0.54 g l^{-1}), concentrations of atrazine all decreased from 4.43 g l^{-1} to below 0.40 g l^{-1} after 3 days treatment. Compared with the partial inhibition of $(NH_4)_2SO_4$ and NH_4NO_3 on 200 mg l^{-1} atrazine medium, the suppressions of $(NH_4)_2SO_4$ and NH_4NO_3 was mostly decreased when 5 g l^{-1} atrazine existed.

Figure 6 suggested that atrazine was degraded quickly both in medium with atrazine as the sole nitrogen source and in medium urea amended as additional nitrogen source. Simultaneously, obviously bacterial growth of the consortium was both observed in the two cultures. After 12 h incubation, cultures reached to the stationary phase and OD_{620} was 1.21 and 1.61, respectively. After 24 h

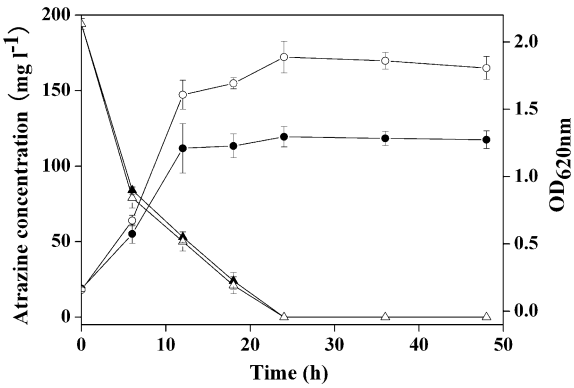

Fig. 6 Atrazine degradation curves by the consortium when urea amended as exogenous nitrogen source. *Symbols*: atrazine residue with urea and atrazine as nitrogen source (△); atrazine residue with atrazine as nitrogen source (▲); OD_{620nm} with urea and atrazine as nitrogen source (○); OD_{620nm} with atrazine as nitrogen sources (●)

inoculation, atrazine were completely mineralized both in atrazine medium and in urea amendment medium, respectively. Compared with the growth in atrazine medium, more biomass of the consortium has been obtained in the cultures when urea as an additional nitrogen source.

Discussion

In present study, the isolated consortium was simple and consisted of two isolates of *Klebsiella* sp. A1 and *Comamonas* sp. A2. Bacteria of different genera are often thought to aid with each other via metabolic cross-feeding and gene transfer, especially in some toxic organic compounds degradation (de Souza et al. 1998a). There are some reports of consortia in which metabolites produced by one strain are utilized by others. In present study, preliminary study suggested that *Klebsiella* sp. A1 could use atrazine as the sole carbon and nitrogen source, but the degrading activity was suppressed by a product of N-ethylammelide which detected by HPLC-MS (data not shown). Another member of *Comamonas* sp. A2 could not use atrazine but N-ethylammelide as the sole carbon and nitrogen source, which not only obtained substrate for its growth but also eliminated product inhibition of N-ehtylammelide in *Klebsiella* sp. A1. As a result, the metabolic collaboration results in the consortium's high atrazine-degrading efficiency when atrazine served as the sole carbon and nitrogen source. More thorough and deeply investigation would be conducted to elucidate the collaboration mechanism in further study.

One member of strain A1 could utilize atrazine as the sole carbon and nitrogen source. To our knowledge, it was the first report that a member of genera *Klebsiella* strain in atrazine degradation. However, no amplification products were obtained from *Klebsiella* sp. A1 using the primers of *trzN*, *atzA* and *atzB* genes, the well known reported genes responsible for the first two steps in atrazine degradation. Southern hybridization further confirmed that the isolate doesn't contain the highly distributed genes of *atzA* and *atzB* and novel enzymes may exist in the strain. This demonstrated the gene diversity of atrazine metabolism in different genera, more thorough molecular analysis will be necessary to elucidate the atrazine-degrading genes in this isolate.

PCR results demonstrated that *Comamonas* sp. strain A2 contains all of the key degrading genes of *atzA, B, C, D, E, F* reported in *Pseudomonas* sp. ADP and showed high identities with them. These sequence homologies were in agreement with the conclusion of de Souza et al. (1998b) that the atrazine metabolism genes of *atzABC* are widely spread and highly conserved in different isolates. The genes distribution in the consortium suggested the highly conservative properties in some genera as well as the diversity among different genera both exist in nature.

The presence of a readily accessible N-source would inhibit atrazine mineralization for the reason of the ring-N atoms of atrazine can be utilized as an N-source by some microorganisms. But to our knowledge, few reports emphasized on the effects of exogenous nitrogen on atrazine degradation (Rhine et al. 2003). Despite of the report by Struthers et al. (1998), in which the isolate of *Agrobacterium radiobacter* J14a was a nonresponsive strain, 50 μg ml^{-1} atrazine was completely degraded in the presence of KNO_3, $(NH_4)_2SO_4$ or NH_4NO_3. The other related reports suggested that ammonium was preferred over atrazine as nitrogen source by certain bacteria as well as soil communities (Abdelhafid et al. 2000a; Rhine et al. 2003). Such as *Pseudomonas* sp. strain ADP and isolate M91-3 (Radosevich et al. 1995; Gebendinger and Radosevich 1999; Garcia-Gonzalez et al. 2003). In present study, the consortium was found insensitive to most of studied exogenous nitrogen sources, low

concentration of 200 mg l^{-1} or high concentration of 5 g l^{-1} atrazine were all mostly consumed despite of the presence of urea, $(NH_4)_2CO_3$, $(NH_4)_2HPO_4$ and NH_4Cl. The partial inhibition of $(NH_4)_2SO_4$ and NH_4NO_3 to 200 mg l^{-1} atrazine degradation has not been observed in high atrazine concentration. Such difference might suggest the positive induction of high atrazine concentration to atrazine degradation enzymes.

Urea is frequently used as a fertilizer in agriculture and often considered as an ideal source to investigate the influence of exogenous nitrogen on atrazine degradation. In present study, atrazine was completely mineralized and coincided with apparent bacteria growth in urea amendment mixture (Fig. 6). The better growth in urea medium (the OD_{620nm} was 1.21 in AT medium and 1.61 in AT + urea) suggested that urea has a positive effect on accelerating bacteria growth while no negative influence on atrazine degradation. Such insensitivity neither agree with some reports in which atrazine mineralization and growth of microorganisms were inhibited by exogenous nitrogen sources (Abdelhafid et al. 2000a; Rhine et al. 2003), nor with the report that fungi biomass was stimulated but atrazine mineralization was suppressed (Entry et al. 1993).

Conclusions

Different from many other reported consortia, the isolated consortium is a good controllable one with simple compositions of two bacteria. Together with its high degrading ability and insensitivity to some nitrogenous fertilizers, the consortium showed good potential in atrazine biodegradation.

Acknowledgments This work was partially supported by the grants from National Natural Science Foundation of China (grant numbers: 30821005 and 20777051), National High Technology Research and Development Program of China (grant numbers: 2007AA061101 and 2007AA10Z401) and National Basic Research Program of China (grant number: 2009CB118906).

References

Abdelhafid R, Houot S, Barriuso E (2000a) Dependence of atrazine degradation on C and N availability in adapted and non-adapted soils. Soil Biol Biochem 32:389–401

Abdelhafid R, Houot S, Barriuso E (2000b) How increasing availabilities of carbon and nitrogen affect atrazine behaviour in soils. Biol Fertil Soils 30:333–340

Alvey S, Crowley DE (1995) Influence of organic amendments on biodegradation of atrazine as a nitrogen-source. J Environ Qual 24:1156–1162

Assaf NA, Turco RF (1994) Accelerated biodegradation of atrazine by a microbial consortium is possible in culture and soil. Biodegradation 5:29–35

Bergey DH, Holt JG (1994) Bergey's manual of determinative bacteriology, 9th edn. Williams & Wilkins, Baltmore

Bouquard C, Ouazzani J, Prome JC, MichelBriand Y, Plesiat P (1997) Dechlorination of atrazine by a *Rhizobium* sp. isolate. Appl Environ Microbiol 63:862–866

de Souza ML, Newcombe D, Alvey S, Crowley DE, Hay A, Sadowsky MJ, Wackett LP (1998a) Molecular basis of a bacterial consortium: interspecies catabolism of atrazine. Appl Environ Microbiol 64:178–184

de Souza ML, Seffernick J, Martinez B, Sadowsky MJ, Wackett LP (1998b) The atrazine catabolism genes *atzABC* are widespread and highly conserved. J Bacteriol 180:1951–1954

de Souza ML, Wackett LP, Sadowsky MJ (1998c) The *atzABC* genes encoding atrazine catabolism are located on a self-transmissible plasmid in *Pseudomonas* sp. strain ADP. Appl Environ Microbiol 64:2323–2326

Eaton RW, Karns JS (1991) Cloning and analysis of *s*-triazine catabolic genes from *Pseudomonas* sp. strain NRRLB-12227. J Bacteriol 173:1215–1222

Entry JA, Mattson KG, Emmingham WH (1993) The influence of nitrogen on atrazine and 2,4-dichlorophenoxyacetic acid mineralization in grassland soils. Biol Fertil Soils 16:179–182

Garcia-Gonzalez V, Govantes F, Shaw LJ, Burns RG, Santero E (2003) Nitrogen control of atrazine utilization in *Pseudomonas* sp. strain ADP. Appl Environ Microbiol 69:6987–6993

Gebendinger N, Radosevich M (1999) Inhibition of atrazine degradation by cyanazine and exogenous nitrogen in bacterial isolate M91–3. Appl Microbiol Biotechnol 51:375–381

Mandelbaum RT, Wackett LP, Allan DL (1993) Mineralization of the s-triazine ring of atrazine by stable bacterial mixed cultures. Appl Environ Microbiol 59:1695–1701

Mandelbaum RT, Allan DL, Wackett LP (1995) Isolation and characterization of a *Pseudomonas* sp. that mineralizes the s-triazine herbicide atrazine. Appl Environ Microbiol 61:1451–1457

Mulbry WW, Zhu H, Nour SM, Topp E (2002) The triazine hydrolase gene *trzN* from *Nocardioides* sp. strain C190: cloning and construction of gene-specific primers. FEMS Microbiol Lett 206:75–79

Piutti S, Semon E, Landry D, Hartmann A, Dousset S, Lichtfouse E, Topp E, Soulas G, Martin-Laurent F (2003) Isolation and characterisation of *Nocardioides* sp. SP12, an atrazine-degrading bacterial strain possessing the gene *trzN* from bulk and maize rhizosphere soil. FEMS Microbiol Lett 221:111–117

Radosevich M, Traina SJ, Hao YL, Tuovinen OH (1995) Degradation and mineralization of atrazine by a soil bacterial isolate. Appl Environ Microbiol 61:297–302

Rhine ED, Fuhrmann JJ, Radosevich M (2003) Microbial community responses to atrazine exposure and nutrient availability: linking degradation capacity to community structure. Microb Ecol 46:145–160

Rousseaux S, Hartmann A, Soulas G (2001) Isolation and characterisation of new Gram-negative and Gram-positive atrazine degrading bacteria from different French soils. FEMS Microbiol Ecol 36:211–222

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual, 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor

Schiavon M (1988) Studies of the leaching of atrazine, of its chlorinated derivatives, and of hydroxyatrazine from soil using C-14 ring-labeled compounds under outdoor conditions. Ecotoxicol Environ Saf 15:46–54

Smith D, Alvey S, Crowley DE (2005) Cooperative catabolic pathways within an atrazine-degrading enrichment culture isolated from soil. FEMS Microbiol Ecol 53:265–273

Strong LC, Rosendahl C, Johnson G, Sadowsky MJ, Wackett LP (2002) *Arthrobacter aurescens* TC1 metabolizes diverse *s*-triazine ring compounds. Appl Environ Microbiol 68:5973–5980

Struthers JK, Jayachandran K, Moorman TB (1998) Biodegradation of atrazine by *Agrobacterium radiobacter* J14a and use of this strain in bioremediation of contaminated soil. Appl Environ Microbiol 64:3368–3375

Topp E, Mulbry WM, Zhu H, Nour SM, Cuppels D (2000) Characterization of *s*-triazine herbicide metabolism by a *Nocardioides* sp. isolated from agricultural soils. Appl Environ Microbiol 66:3134–3141

Weightman A, Slater J (1988). The problem of xenobiotics and recalcitrance. In: Lynch J, Hobbie J (eds) Microorganisms in action: concepts and applications in microbial ecology. Blackwell, Oxford, pp 322–347

Yanzekontchou C, Gschwind N (1994) Mineralization of the herbicide atrazine as a carbon source by a *Pseudomonas* strain. Appl Environ Microbiol 60:4297–4302