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Original Research

Isolating and Identifying the Atrazine-Degrading Strain *Arthrobacter* sp. LY-1 and Applying it for the Bioremediation of Atrazine-Contaminated Soil

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Abstract

Atrazine is a widely used herbicide, and because of its potential to seriously pollute soil and water resources, has attracted widespread attention. In this study, the bacterial strain LY-1 was isolated and identified as the species *Arthrobacter*. At present, there are many different atrazine-degrading bacteria that have been screened out, including *Arthrobacter* sp., *Pseudomonas* sp., and *Shewanella* sp. However, previous reports only studied their degradation effects and soil remediation capabilities under optimum conditions and there were few studies performed which took into account a wider array of environmental circumstances. This study investigated the degradation effects of LY-1, as well as its capacity for soil remediation, under various conditions. The strain had broad optimum ranges of temperature and pH and the additional carbon and nitrogen sources did not decrease the atrazine degradation rate. In addition, the soil remediation tests indicated that the strain LY-1 might be a good candidate for bioremediation of atrazine-polluted soil.

Keywords: atrazine, *Arthrobacter* sp., atrazine-degrading genes, biodegradation, bioremediation

Introduction

The s-triazine chemical herbicide atrazine (2-chloro-4-ethylamino-6-isopropylamino-1, 3, 5-triazine) is widely used to control annual grass and broadleaf weeds in the agriculture of sugarcane (*Saccharum officinarum*), sorghum (*Sorghum* spp.), maize (*Zea mays*), and other crops [1]. Because of its long half-life and high mobility in soil, atrazine and its derivatives have been detected in soils, and surface and groundwater [2]. Moreover, it also interferes with the endocrine systems of humans and animals and its use affects ecosystems and human health [3]. In recent years, bioremediation methods have become an ideal solution to the problem of atrazine contamination because of their low cost, effectiveness, and lack of secondary pollution [4].

Currently, a number of bacteria with different atrazine degradation efficiencies and growth characteristics have been isolated. These strains include *Shewanella* sp. YJY4, *Arthrobacter* sp. TC1, *Pseudomonas* sp. ADP, *Citricoccus* sp. TT3, *Rhodococcus* sp. BCH2, and *Bacillus subtilis* HB-6 [5-9]. Of all presently known strains, the degradation

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pathways of *Pseudomonas* sp. ADP and *Arthrobacter* sp. TC1 are studied most clearly. Strain ADP contains the degradation genes *atzA*, *atzB*, *atzC, atzD*, *atzE*, and *atzF*, which can completely degrade atrazine [10]. Stain TC1 contains the degradation genes *trzN*, *atzB,* and *atzC*, which can transform atrazine into non-toxic cyanuric acid. The atrazine-degrading genes *trzN*, *atzA*, *trzD,* and *atzD* have the same function respectively [11].

In this study, a high-efficiency atrazine degrader, bacterial strain LY-1, was isolated from a cornfield where atrazine had been used for a long time. The identification of the strain, its degradation genes, the influence of environmental factors, and soil remediation ability were studied. Environmental factors have a significant effect on atrazine degradation [12]. These factors include atrazine concentration, temperature, pH, carbon, and nitrogen sources. The objective of this study was to provide a highly efficient strain resource for the bioremediation of atrazine-contaminated soil.

Materials and Methods

Soil Samples and Reagents

Experimental soil was sampled from the surface layer of the cornfield where atrazine was used for a long time (0–10 cm). The area is located northwest of Heilongjiang Province, China. Soil samples were treated in a 2.0 mm sieve and stored at 4ºC.

Atrazine of 99% purity was used to characterize the atrazine-degrading bacteria, which were purchased from Shanghai KALANG Technology Ltd. The 99% pure cyanuric acid was purchased from WEIFANG KAIRUI Chemical Co., Ltd. All other chemicals in the experiment were analytical or HPLC-grade reagents.

The liquid enrichment medium consisted of mineral salts medium (MSM), 3 $g \cdot l^{-1}$ glucose, and 100 $mg \cdot l^{-1}$ atrazine as the carbon and nitrogen sources, respectively. The MSM contained 0.5 $g \cdot l^{-1}$ of KH_2PO_4, 3.0 $g \cdot l^{-1}$ of K_2HPO_4, 0.2 $g \cdot l^{-1}$ of $MgSO_4 \cdot 7H_2O$, 0.5 $g \cdot l^{-1}$ of NaCl, and 1 $ml \cdot l^{-1}$ of trace element concentrate solution. Trace element concentrate solution had the following composition: $FeSO_4 \cdot 7H_2O$, 1 $g \cdot l^{-1}$; $ZnSO_4 \cdot 7H_2O$, 5 $g \cdot l^{-1}$; $CuSO_4 \cdot 5H_2O$, 0.4 $g \cdot l^{-1}$; $MnSO_4 \cdot H_2O$, 1 $g \cdot l^{-1}$; EDTA, 2.5 $g \cdot l^{-1}$; $Na_2MoO_4 \cdot 2H_2O$, 0.25 $g \cdot l^{-1}$, and $Na_2B_4O_7 \cdot 10H_2O$, 0.2 $g \cdot l^{-1}$. The solid medium was added to 13 $g \cdot l^{-1}$ of agar. All of the above cultures were regulated to pH 7.0 and sterilized at 121ºC for 30 min.

Enrichment and Isolation

The five-gram soil specimens were transferred to 250 ml flasks containing 100 ml of MSM, to which 3 $g \cdot l^{-1}$ glucose and 100 $mg \cdot l^{-1}$ atrazine were added. These specimens were cultured on a 160 $r \cdot min^{-1}$ shaker for 7 days and the temperature was kept at 30ºC. And then enrichment culture was inoculated into the new medium according to the inoculation amount of 5%. The concentration of atrazine in the new medium was 200 $mg \cdot l^{-1}$ and this process was repeated until the concentration of atrazine reached 500 $mg \cdot l^{-1}$. Eventually, the medium was diluted and placed onto the agar plates that contained 100 $mg \cdot l^{-1}$ of atrazine. LY-1 was used for further studies because it demonstrated a high capacity for degrading atrazine.

Identifying Atrazine-Degrading Strain LY-1

The identification of strain LY-1 was accomplished by observing the results of biochemical reactions and 16S rDNA sequencing. The total DNA of the strain was used as a template for amplification. 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-TACGGCTACCTTGTTACGACTT-3′) were used as forward and reverse primers, respectively [13]. The PCR conditions were as follows: 94ºC for 5 min followed by 30 cycles of 94°C for 1 min, 54ºC for 1 min, and 72ºC for 2 min, with a final extension of 10 min at 72ºC. The products of PCR were divided on a 1.2% agarose gel electrophoresis. The resulting DNA fragments were purified through AxyPrep DNA gel extraction kit (Axygen), and then cloned into the pMD19-T vector (TaKaRa, China). The vector was transformed into *Escherichia coli* strain DH5α. Genetic sequencing was completed by Jilin Comate Bioscience Co., Ltd., and the results were compared using the Blast program in the NCBI GenBank nucleotide database. MEGA version 6.0 was used to construct the phylogenetic tree.

PCR Detection of Atrazine-Degrading Genes

Currently known atrazine-degrading genes included *atzA, atzB, atzC, atzD, atzE, atzF, trzD,* and *trzN* [14]. The size, sequences, and anneal temperature of these primers were shown in Table 1. The total chromosomal DNA of strain LY-I was used as a template to amplify these genes. The conditions of PCR were as follows: at 94ºC preheated 5 min, 30 cycles of denaturation at 94ºC for 1 min, annealed at 58, 64, 54, 60, 52, 55, 56, and 53ºC for 1 min to *atzA, atzB, atzC, atzD, atzE, atzF* genes and *trzN, trzD* genes, respectively, extension at 72ºC for 90 s, and final extension at 72ºC for 10 min. The research team performed sequencing and sequence analysis according to the above method.

Growth and Biodegradation Capacity of Strain LY-1 and Influential Factors

In order to determine the growth curve of the strain, either atrazine or cyanuric acid (100 $mg \cdot l^{-1}$) was added to the 100 ml MSM medium as the nitrogen source, and glucose (3 $g \cdot l^{-1}$) was added as the carbon source. The strain LY-1 used for inoculation was incubated at 30ºC for 12 h and centrifuged at 7000 $r \cdot min^{-1}$, and it was suspended with sterile water to 0.1 at 600 nm (OD_{600}). One milliliter of treated bacteria was inoculated into the medium and these treatments were incubated in

Table 1. PCR primers, expected product sizes, and annealing temperatures in the amplification of atrazine-degrading genes.

Gene	Primer	Gene size (kb)	Sequence (5'→3')	Annealing (ºC)
atzA	*atzA*-1	530	CCATGTGAACCAGATCCT	58
	atzA-2		TGAAGCGTCCACATTACC	
atzB	*atzB*-1	510	TCACCGGGGATGTCGCGGGC	64
	atzB-2		CTCTCCCGCATGGCATCGGG	
atzC	*atzC*-1	610	GCTCACATGCAGGTACTCCA	54
	atzC-2		GTACCATATCACCGTTTGCCA	
atzD	*atzD*-1	1050	GGAGACATCATATGTATCACATCGACGTTTTC	60
	atzD-2		CCAATAAGCTTAGCGCGGGCAATGACTGCA	
atzE	*atzE*-1	990	TACGCGGTAAAGAATCTGTT	52
	atzE-2		GGAGACCGGCTGAGTGAGA	
atzF	*arzF*-1	910	CGATCGGAAAAACGAACCTC	55
	atzF-2		CGATCGCCCCATCTTCGAAC	
trzD	*trzD*-1	760	CCTCGCGTTCAAGGTCTACT	53
	trzD-2		TCGAAGCGATAACTGCATTG	
trzN	*trzN*-1	1360	ATGATCCTGATCCGCGGACTGA	56
	trzN-2		CTACAAGTTCTTGGGAATGAGTG	

a 160 r·min^{-1} shaker with a culture temperature of 30ºC. Samples were taken every 4 h, until 48 h worth of samples were measured. The growth of the strain was determined by measuring its absorbance at 600 nm using a spectrophotometer (U-2910; HITACHI, Japan). The effects of atrazine concentration, temperature, pH, carbon, and nitrogen sources on the growth of the strain were also measured. All experiments were repeated three times to ensure accuracy.

When the effect of atrazine concentration on the growth of the strain was determined, the concentration was adjusted to 50, 100, 200, 500 and 1000 mg·l^{-1}, respectively. The temperature was adjusted to 5, 10, 15, 20, 25, 30, 35, and 40°C and the pH was adjusted to 3, 4, 5, 6, 7, 8, 9, 10 and 11, respectively. The concentration of atrazine in these cultures was 100 mg·l^{-1}. The effects of the carbon and nitrogen sources on the growth of strain were studied by referring to previous methods [15]. The methods for determining the effects of carbon sources on the growth of strain LY-1 were as follows: 1) MSM (100 ml) + 100 mg·l^{-1} KNO_3 +100 mg·l^{-1} atrazine; 2) MSM (100 ml) + 100 mg·l^{-1} KNO_3 + 3 g·l^{-1} glucose; 3) MSM (100 ml) + 100 mg·l^{-1} KNO_3 + 3 g·l^{-1} sucrose; 4) MSM (100 ml) + 100 mg·l^{-1} KNO_3 + 3 g·l^{-1} starch; and 5) MSM (100 ml) + 100 mg·l^{-1} KNO_3. The last one is a control group which did not add a carbon source. The methods for determining the effects of additional nitrogen sources on the growth of strain LY-1 were as follows: 1) MSM (100ml) + 3 g·l^{-1} starch + 100 mg·l^{-1} atrazine + 100 mg·l^{-1} cyanuric acid; 2) MSM (100ml) + 3 g·l^{-1} starch + 100 mg·l^{-1} atrazine + 100 mg·l^{-1} urea; 3) MSM (100ml) + 3 g·l^{-1} starch + 100 mg·l^{-1} atrazine + 100 mg·l^{-1} NH_4Cl; 4) MSM (100ml) + 3 g·l^{-1} starch + 100 mg·l^{-1} atrazine + 100 mg·l^{-1} KNO_3; and 5) MSM (100ml) + 3 g·l^{-1} starch + 100 mg·l^{-1} atrazine. The last one is a control group, which did not add an additional nitrogen source. These treatment groups were incubated in a 160 r·min^{-1} shaker for 48 h, and the culture temperature was maintained at 30ºC. The growth of the strain was determined by measuring its absorbance at 600 nm using a spectrophotometer (U-2910; HITACHI, Japan).

The degradation capacity of atrazine was monitored using high-performance liquid chromatography (HPLC) analysis [16]. The concentrations of atrazine, as well as its temperature and pH, were the same as those mentioned above, and the concentration of atrazine was 100 mg·l^{-1}. The methods for determining the effects of carbon sources on the biodegradation capacity of strain LY-1 were as follows: 1) MSM (100 ml) + 100 mg·l^{-1} atrazine + 3 g·l^{-1} glucose; 2) MSM (100 ml) + 100 mg·l^{-1} atrazine + 3 g·l^{-1} sucrose; 3) MSM (100 ml) + 100 mg·l^{-1} atrazine + 3 g·l^{-1} starch; and 4) MSM (100 ml) + 100 mg·l^{-1} atrazine. The methods for determining the effects of nitrogen sources on the biodegradation capacity of strain LY-1 were as follows: 1) MSM (100 ml) + 3 g·l^{-1} starch + 100 mg·l^{-1} atrazine + 100 mg·l^{-1} urea; 2) MSM (100 ml) + 3 g·l^{-1} starch + 100 mg·l^{-1} atrazine + 100 mg·l^{-1} NH_4Cl; 3) MSM (100 ml) + 3 g·l^{-1} starch + 100 mg·l^{-1} atrazine + 100 mg·l^{-1} KNO_3; and 4) MSM (100 ml) + 3 g·l^{-1} starch + 100 mg·l^{-1} atrazine. In the two

experiments, control groups were set which did not add carbon and additional nitrogen sources. The atrazine concentration and cyanuric acid production were quantified by HPLC (Waters 600; Waters). The detection conditions for atrazine were as follows: the mobile phase was methanol: water = 80:20 (v:v), the flow rate was 1 ml·min^{-1} and the UV detector was set to 216 nm. For the detection of cyanuric acid [17], the flow rate was 0.8 ml·min^{-1} (50 mM K_2HPO_4 / methanol = 95:5, v:v), and the UV detector was set to 213 nm. The injection volumes of all samples were 20 µl.

Bioremediation of Atrazine-Contaminated Soil

The soil was collected from the experimental farm of Northeast Agricultural University, where atrazine had never been used (0-20 cm). Soil samples were treated in a 2.0 mm sieve and sterilized at 121ºC for 50 min. Then, the soil was baked in an oven (80ºC) and stored at 4ºC until used.

The atrazine was dissolved in methanol (10^4 mg·l^{-1}). The soil was placed in a fume hood for 48 h until the methanol evaporated. The final concentration of atrazine in the soil was 100 mg·kg^{-1}. 200 g of contaminated soil was added to each pot. The strain LY-1 was resuspended with sterile water to a density of about 1×10^6 and 1×10^8 colony forming units (CFU)·ml^{-1} (adjusted using a standard curve relating absorbance at 600 nm with plate count on MSM) [18]. Experimental treatments were used as follows: 1) contaminated soil (200 g) + strain LY-1(5 ml, 1×10^6 CFU·ml^{-1}); 2) contaminated soil (200 g) + strain LY-1(5 ml, 1×10^8 CFU·ml^{-1}); and 3) contaminated soil (200 g) + sterile water (5 ml). Each treatment was performed in triplicate. During the test period, the soil-water content of each treatment was maintained at 20%. All treatments were placed at 25ºC room temperature. On the 7th and 14th days, 10 g of soil samples were weighed from each group to detect the concentration of residual atrazine.

Thirty milliliters of methanol was added to each weighed soil sample, which was agitated for 2 h. The samples were placed for 24 h and then shaken for another hour. The treatments were centrifuged at 8000 r·min^{-1} for 10 min and the supernatant was passed through a 0.22 µm filter for the detection of HPLC. The detection conditions were the same as those mentioned above.

Results and Discussion

Isolation and Identification of Strain LY-1

The strain LY-1 was isolated from the experimental soil, which was a Gram-positive bacterium. The results of HPLC showed that the strain could degrade 99.5% of the atrazine (100 mg·l^{-1}) within 48 h. The 16S rDNA amplification products were sequenced and the sequence was submitted to GenBank (under accession No. KX905053). The sequence had a high similarity with many *Arthrobacter* sp. strains (Fig. 1). According to its physiological and biochemical characteristics, together with the sequential analysis, the strain LY-1 was identified as *Arthrobacter* species. *Arthrobacter* species strains were often found in many contaminated areas, and many *Arthrobacter* species were isolated from agricultural soil and were capable of degrading atrazine [19]. These results showed that *Arthrobacter* sp. LY-1 might hold great potential for the bioremediation of atrazine-contaminated soil.

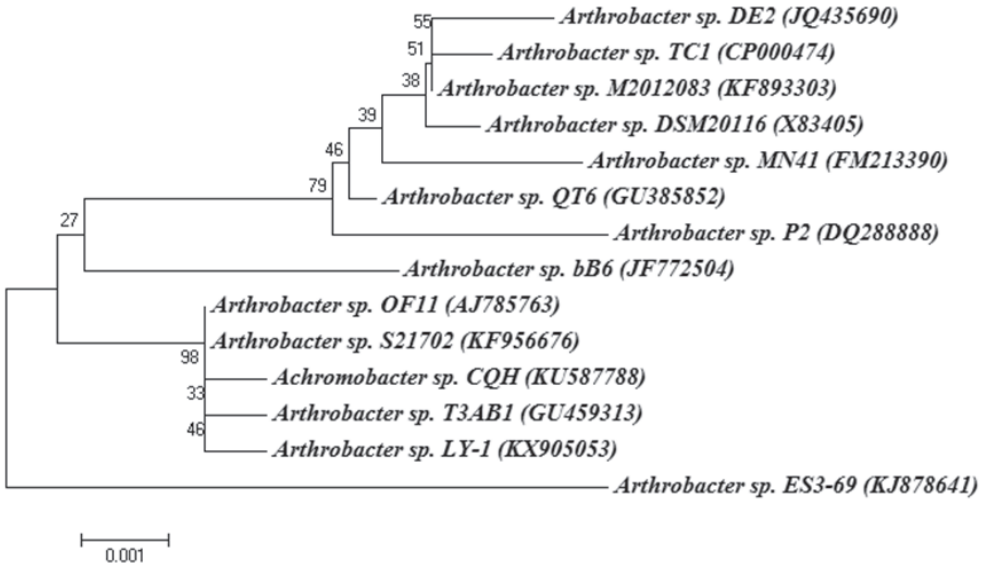

Fig. 1. Phylogenetic analysis of the 16S rDNA sequences of *Arthrobacter* sp. LY-1; numbers on the branch points represent bootstrap values from the neighborhood-joining analysis of 1,000 resampled data sets; the bar indicates 0.1% substitution per nucleotide position.

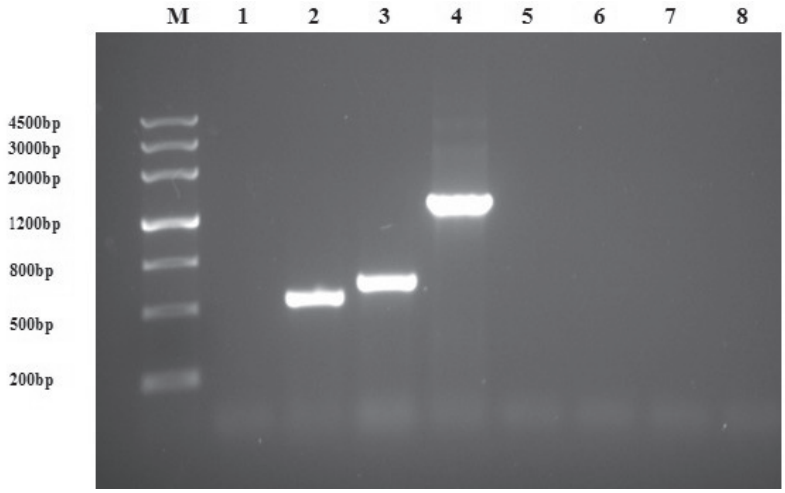

Fig. 2. Agarose gel electrophoresis (1.2%) of PCR products of atrazine-degrading genes of strain LY-1; lanes 1 to 8 indicate the PCR amplification products for *atzA, atzB, atzC, trzN, atzD, atzE, atzF, and trzD.*

PCR Detection of Atrazine-Degrading Genes in Strain LY-1

The atrazine-degrading related genes were amplified by using the total DNA of the strain LY-1 as template. The products of 1370 bp *trzN*, 500 bp *atzB* and 600 bp *atzC* genes were obtained, as shown in Fig. 2, while the products of other genes were not obtained, including *atzC*, *atzD*, *atzE*, *atzF*, and *trzD*. The conditions of PCR were adjusted repeatedly, but other atrazine-degrading genes were not amplified successfully. The results of sequence determination and comparison demonstrated that the nucleotide sequences of *trzN*, *atzB*, and *atzC* from *Arthrobacter* sp. LY-1 showed 98%, 99%, and 100% sequence similarity with those of *Arthrobacter* sp. TC1 (Genbank accession number CP000475). These all indicated that strain LY-1 contained *trzN*, *atzB*, and *atzC* genes.

A number of reported *Arthrobacter* species only contained *trzN*, *atzB*, and *atzC* genes, such as *Arthrobacter* sp. DAT1, *Arthrobacter* sp. AK-YN10 and *Arthrobacter* sp. TC1 [20-22]. These *Arthrobacter* species could only degrade atrazine to cyanuric acid and it was supposed that the strain LY-1 could also degrade atrazine to non-toxic cyanuric acid.

Growth and Degradation Characteristics of Strain LY-1

The growth characteristics of strain LY-1 were measured in the medium in which the sole nitrogen sources were atrazine or cyanuric acid (Fig. 3a). When using atrazine as the sole nitrogen source, OD_{600} increased markedly and reached its maximum value at 44 h, while within 12 h after inoculation the strain grew slowly, indicating that the strain has a certain period of adaptation. When cyanuric acid was used as the sole nitrogen source, the strain hardly grew. This illustrated that strain LY-1 could not utilize cyanuric acid as the sole nitrogen source to grow.

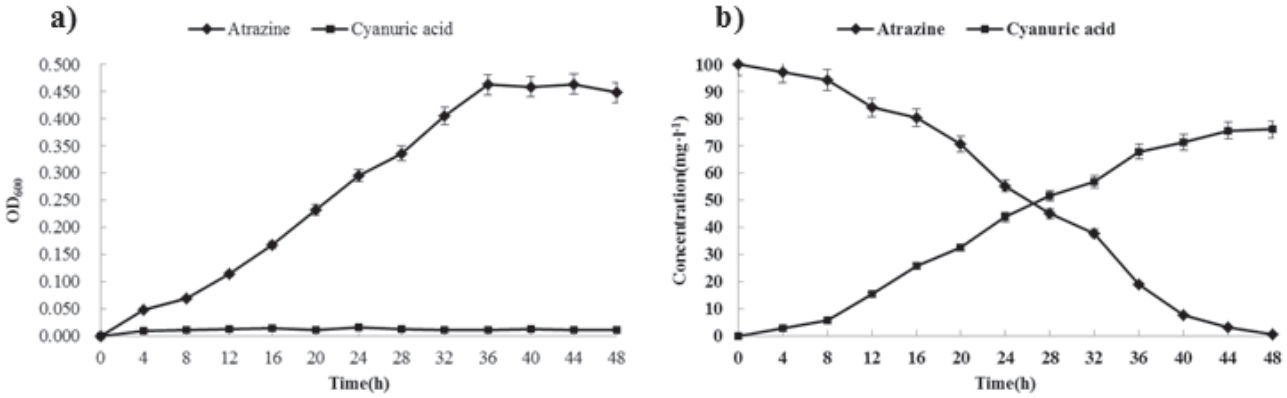

Fig. 3. a) Growth curve of strain LY-1 in MSM medium containing either atrazine or cyanuric acid (100 mg·l^{-1}) as the sole nitrogen source and b) Atrazine degradation curve containing atrazine (100 mg·l^{-1}) as the sole nitrogen source; the concentration of cyanuric acid was detected by HPLC in MSM medium.

We also measured the degradation characteristics of strain LY-1 in the medium in which the sole nitrogen source was atrazine (Fig. 3b)). The strain LY-1 could degrade 99.5% of the atrazine (100 mg·l^{-1}) within 48 h. With the decrease of atrazine concentration, the concentration of cyanuric acid increased gradually.

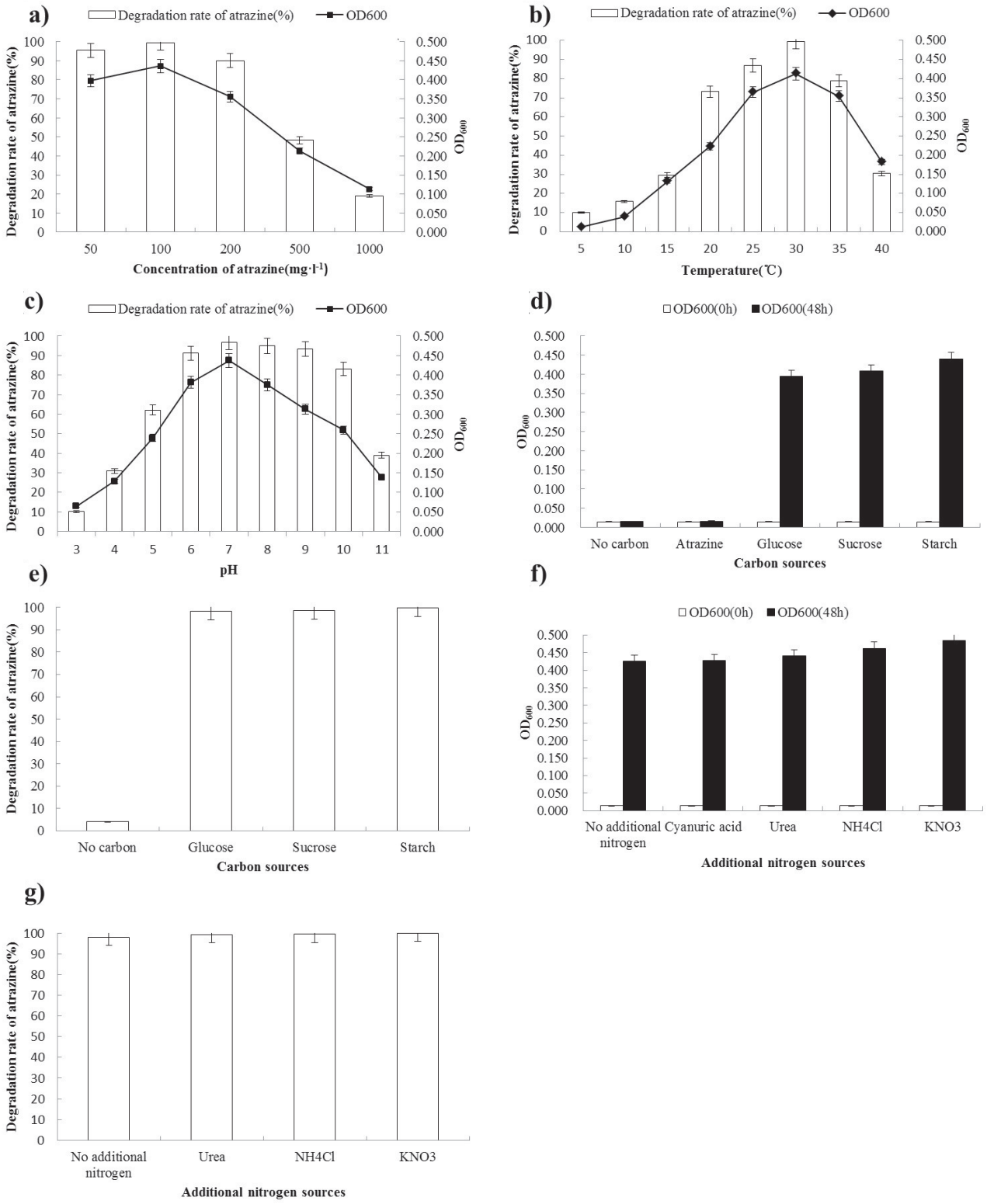

Fig. 4. After 48-h incubation: a) Effects of atrazine concentration on growth and degradation capacity of strain LY-1, b) effects of temperature on the growth and the degradation capacity of strain LY-1, c) effects of pH on the growth and the degradation capacity of strain LY-1, d) effects of different carbon sources on the growth of strain LY-1, e) effects of different carbon sources on the degradation capacity of strain LY-1, f) effects of different additional nitrogen sources on the growth of strain LY-1, and g) effects of different additional nitrogen sources on the degradation capacity of strain LY-1.

At 44-48 h, atrazine was almost completely degraded and the concentration of cyanuric acid was also stable. All of these certified that *Arthrobacter* sp. LY-1 could convert atrazine to cyanuric acid. The speculative pathway of atrazine degradation by *Arthrobacter* sp. LY-1 was the same as the reported pathway [23].

Effects of Environmental Factors on Growth and Biodegradation

Atrazine concentration, temperature, pH, carbon, and nitrogen sources influence atrazine degradation, but few reported studies have investigated the effects of these conditions on atrazine degradation. Most the previous studies referred to the degradation of atrazine at lower concentrations. *Arthrobacter* sp. C3 could almost completely degrade atrazine (25 $mg \cdot l^{-1}$) after being cultured for 30 h and *Arthrobacter* species DNS10 could degrade 99.6% of 100 $mg \cdot l^{-1}$ of atrazine within 36 h [24-25]. Fig. 4 indicated that when the atrazine concentration was below 200 $mg \cdot l^{-1}$, the degradation rate of atrazine in the medium was more than 90% within 48 h. When atrazine concentration was 500 $mg \cdot l^{-1}$, the degradation rate was 50%. This showed that the strain demonstrated a positive degradation effect, although the atrazine concentration was higher. When the atrazine concentration was 100 $mg \cdot l^{-1}$, the growth and degradation abilities of strain LY-1 were optimal. When the atrazine concentration was 1000 $mg \cdot l^{-1}$, the growth and degradation abilities of strain LY-1 were less effective. Atrazine has a toxic effect on microorganisms, and if the concentration of atrazine in the culture is too high, then its toxicity will inhibit the metabolic activities of microorganisms, resulting in a significant reduction in growth and degradation activity.

The currently known optimum temperature range for degradation of atrazine by *Arthrobacter* species HB-5 is 20-40ºC [26]. The effects of different temperatures on growth and atrazine degradation by *Arthrobacter* species LY-1 were shown in Fig. 4b), which indicated that the optimum temperature range was 20-35ºC. Growth and degradation abilities were both inhibited when the temperature was higher than 35ºC or lower than 20ºC. Fig. 4b also demonstrated that strain LY-1 exhibited a particular degradation ability at low temperatures, and the degradation rate of atrazine was about 30% at 15ºC. Therefore, strain LY-1 was also suitable for bioremediation of atrazine at lower temperatures.

The optimum pH range for atrazine degradation by *Pseudomonas* sp. ZXY-1 was 6.0-8.0 and *Arthrobacter* species HB-5 was 6.0-9.0 [27]. The effects of different pH values on the growth of *Arthrobacter* species LY-1 and its atrazine degradation ability were shown in Fig. 4c). The optimum pH range was 6.0–10.0 and the degradation and growth abilities of the strain under alkaline conditions were better than those in acidic conditions. The strain still could have degradation rates of 62.1% and 38.9% at pH 5.0 and 11.0, respectively. These results indicated that strain LY-1 had wide ranges of temperature and pH, and it demonstrated better environmental adaptability and scavenging ability in contaminated soil. Overall, the strain had superior bioremediation potential.

Carbon sources were a necessary precondition for the growth of strain LY-1. Fig. 4d) indicated that starch was the most suitable carbon source for its growth. When glucose, sucrose, and starch were used as carbon sources, the removal rates of atrazine were more than 95%; however, when no carbon source was added, the concentration of atrazine changed insignificantly (Fig. 4e). Moreover, when atrazine was used as the sole carbon source, the strain hardly grew. All these findings point to the fact that atrazine could not be used as the sole carbon source of strain LY-1.

Fig. 4f) demonstrated the effects of additional nitrogen sources on the growth of strain LY-1. The addition of urea, ammonium salt, and nitrate promoted the growth of the strain LY-1, with nitrate resulting in the highest strain growth, while cyanuric acid did not cause higher growth. These results implied that strain LY-1 could not use cyanuric acid as a nitrogen source to grow. This also indirectly verified that the strain could convert atrazine to cyanuric acid, but it was unable to degrade cyanuric acid. Some previous studies showed that additional nitrogen sources had a negative impact on atrazine degradation [28-30]. While additional nitrogen sources did not affect the degradation of atrazine by strain LY-1 (Fig. 4g), the degradation rates all exceeded 98%. This illustrated that strain LY-1 had a great potential for bioremediation of atrazine-contaminated soil, and even contaminated soil containing rich nitrogen sources.

Bioremediation Effect of Atrazine-Contaminated Soil by Strain LY-1

The degradation rate of atrazine in the soil by strain LY-1 was shown in Fig. 5. After incubation at 25ºC for

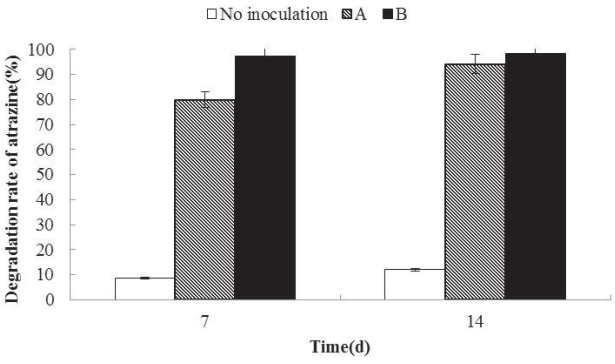

Fig. 5. The degradation rate of atrazine in the soil by strain LY-1 after incubation at 25°C for 7 d and 14 d: A) strain LY-1 was resuspended with sterile water to a density of about 1×10^6 colony forming units (CFU)$\cdot ml^{-1}$ and B) strain LY-1 was resuspended with sterile water to a density of about 1×10^8 CFU$\cdot ml^{-1}$.

7 d, 79.9% and 97.4% of atrazine were removed by 1×10^6 CFU·ml^{-1} and 1×10^8 CFU·ml^{-1} of LY-1, respectively. After incubation at 25°C for 14 d, the degradation rates of atrazine by 1×10^6 CFU·ml^{-1} and 1×10^8 CFU·ml^{-1} of LY-1 were 94.1% and 98.3%, respectively. However, after incubation at 25ºC for 7 d and 14 d, 8.7% and 12% of atrazine were removed in the negative control. In comparison, after 20 days, atrazine degradation rates by *Arthrobacter* sp. DNS10 were 67.7%, and the initial concentration of atrazine in the soil was 20 mg·kg^{-1}. The study showed that atrazine-degrading bacteria *Arthrobacter* sp. LY-1 had a better remediation capability for atrazine-contaminated soil compared with most other reported atrazine-degrading strains. This strain could achieve a better degradation effect in a relatively short time and might be a new candidate for the remediation of atrazine-contaminated soil.

Conclusions

A highly efficient atrazine-degrading bacterial strain, *Arthrobacter* sp. LY-1, was isolated from agricultural soil. The strain harbored *trzN*, *atzB*, and *atzC* genes, which could transform atrazine to cyanuric acid. The strain was capable of using atrazine as the sole nitrogen source to grow, while atrazine could not be used as a carbon source for growth. The degradation rates of atrazine under different environmental factors were determined and when atrazine concentration was 100 mg·l^{-1}, the growth and degradation ability of strain LY-1 were the best. The optimum temperature range of this strain was 20-35ºC, and the optimum pH range was 6.0-10.0. Starch was the most suitable carbon source for its growth and atrazine degradation. Additional nitrogen sources did not affect the degradation of atrazine. Strain LY-1 could degrade 99.5% of atrazine (100 mg·l^{-1}) in the medium within 48 h, and in the soil, atrazine (100 mg·kg^{-1}) was almost completely degraded by the strain after 14 days. These results demonstrated that strain LY-1 held promising potential for bioremediation of atrazine-contaminated soil.

Acknowledgements

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Conflict of Interest

The authors declare that they have no conflict of interest.

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