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SHORT RESEARCH AND DISCUSSION ARTICLE

# Combined bioremediation of atrazine-contaminated soil by *Pennisetum* and *Arthrobacter* sp. strain DNS10

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**Abstract** Strain DNS10 was isolated from the black soil collected from the northeast of China which had been cultivated with atrazine as the sole nitrogen source. *Pennisetum* is a common plant in Heilongjiang Province of China. The main objective of this paper was to evaluate the efficiency of plant–microbe joint interactions (*Arthrobacter* sp. DNS10 + *Pennisetum*) in atrazine degradation compared with single-strain and single-plant effects. Plant–microbe joint interactions degraded 98.10 % of the atrazine, while single strain and single plant only degraded 87.38 and 66.71 % after a 30-day experimental period, respectively. The results indicated that plant–microbe joint interactions had a better degradation effect. Meanwhile, we found that plant–microbe joint interactions showed a higher microbial diversity. The results of microbial diversity illustrated that the positive effects of cropping could improve soil microbial growth and activity. In addition, we planted atrazine-sensitive plants (soybean) in the soil after repair. The results showed that soybean growth in soil previously treated with the plant–microbe joint interactions treatment was better compared with other treatments after 20 days of growth. This was further proved that the soil is more conducive for crop cultivation. Hence, plant–microbe joint interactions are considered to be a potential tool in the remediation of atrazine-contaminated soil.

**Keywords** Atrazine (AT) · Enhance bioremediation · *Pennisetum* · Plant–microbe joint interactions · Strain DNS10 · Ecological safety

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## Introduction

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine) is one of the most widely used herbicides in many countries all over the world and in the control of broad-leafed weeds. Atrazine residues in agricultural soils after application have been reported to range from 21 days to over a year according to the soil physical and chemical properties. Due to its high mobility and long half-life in soil, residues of atrazine and its metabolites have been detected in soil, surface water, and groundwater years after application (Zaya et al. 2011). Furthermore, several toxicological researches have reported atrazine's potential to disrupt endocrine hormone metabolism and cause liver cancer; hence, it has been defined as a kind of endocrine disruptor (Moore and Lower 2001; Hayes et al. 2002; Friedmann 2002). For these reasons, atrazine has received a large number of scientific concerns by both governmental and academic investigators (Zaya et al. 2011).

Previous researches have reported that atrazine can undergo degradation through chemical dechlorination and biodegradation processes in the environment (Getenga et al. 2009). Due to its characteristics of low cost and good effect, microbial metabolism has been regarded as the most important mechanism of these metabolic processes (De Souza et al. 1998). Furthermore, bioaugmentation, which is an approach to improve contaminant cleanup through the addition of specific microbial cultures at such a polluted site, may be required when pollutant-degrading microorganisms are not present or where indigenous degraders cannot degrade xenobiotic rapidly (Gentry et al. 2004). Sometimes, the microorganisms also could promote the growth of plants during the pollutant degradation process. Several researches reported that a Gram-negative bacterium *Comamonas* sp. strain KD7 isolated from the root of white clover could degrade dioxin-related compounds and promote the growth of white clover (Wang et al. 2004). In addition, the plants also could promote the pollutants

degrading abilities of the microorganisms. For example, the concentration of total petroleum hydrocarbon (TPH) decreased significantly in the rhizosphere of *Atriplex centralasiatica*, *Scorzonera mongolica*, *Maxim*, and *Limonium bicolor* (Xu et al. 2011). To date, many researches have used different isolated bacterial strains to metabolize atrazine, but details about combined bioremediation of atrazine-polluted soil by plant and microbes are poorly described.

This paper intends to investigate the bioremediation effect of atrazine-contaminated soil by combination of *Pennisetum* and *Arthrobacter* sp. DNS10. Reduction in atrazine concentrations in the different soil units was used to describe the rate of atrazine degradation by the different treatments applied. The changes of indigenous bacterial variety are also investigated by polymerase chain reaction and denaturing gradient gel electrophoresis (PCR–DGGE) in order to insure the ecological safety of the bioremediation process. Finally, the bioremediation efficiency was evaluated by using soybean, an atrazine-sensitive plant. All the results achieved in this paper would help to improve previous understanding about the important role played by typical plants in combined remediation processes.

## Materials and methods

### Chemicals

Atrazine (>97 % purity) was gratefully donated by Shandong Pesticide Research Institute, China. All other chemicals in the experiment were chemical grade or analytical grade.

### Atrazine-degrading bacteria and atrazine-resistant plant

The bacterial strain DNS10 studied in this paper was purified from an atrazine-degrading consortium which was isolated from the black soil used in our previous work. In addition, our previous research showed that the strain DNS10 was the sole member in the consortium which utilized atrazine as the sole nitrogen source for growth (Zhang et al. 2011). The test plant *Pennisetum* is a common plant in Heilongjiang Province of China. Our previous work also showed that *Pennisetum* had dominant atrazine resistance compared to other common herbaceous plants, such as Sudan grass and ryegrass.

### Experimental soil

The experimental soil, the typical black soil, was sampled from the Xiangfang Experimental Farm located in the south part of Heilongjiang Province, China. The soil total nitrogen and total phosphorus contents are 1.13 and 0.52 g $kg^{-1}$, respectively, and the pH of the soil ranged between 6.48 and 6.68. The available nitrogen and potassium contents were 113.59 and 160.23 mg $kg^{-1}$, respectively.

### Bioremediation of atrazine-contaminated soil

To investigate the atrazine-degrading ability of the plant and the microorganism, some experimental treatments were used by different combinations of *Pennisetum* and strain DNS10 as follows: (1) contaminated soil only (treatment 1), (2) contaminated soil + *Pennisetum* only (treatment 2), (3) contaminated soil + strain DNS10 only (treatment 3), and (4) contaminated soil + *Pennisetum* + strain DNS10 (treatment 4).

To ensure good distribution, atrazine was diluted in acetone (10 mL of acetone per milligram of atrazine) before it was spiked into the soil. In order to allow the acetone to evaporate, the soil was placed in a fume hood for about 24 h. Into the pots containing the corresponding treatments, 500 g of contaminated soil was added, after each pot was sown with 20 seeds of *Pennisetum*. The initial soil atrazine concentration was 19.52 mg $kg^{-1}$.

Soil water content of 25 % was maintained for all experimental units during the experimental period. The pots were placed on the balcony during the day and on the windowsill in the evening. All the samples were kept at 25 °C room temperature. Periodically (5, 10, 15, 20, 25, and 30 days), soil of about 10 g was collected from each unit to monitor the atrazine residual concentration and bacteria diversity of the soil samples.

### Extraction and measurement of atrazine in soil samples

Fifty milliliters of extracting solution (methanol–water=9:1, *v*/*v*) was added into the soil samples (treatments 1–4) collected and left to stand overnight and centrifuged the mixture to collect the supernatant. The volume of the supernatant was condensed to 15 mL by a rotary evaporator at 60 °C. The residual liquid was transferred to a 250-mL separating funnel and then 15 mL of chloroform was added to extract the atrazine. The process of adding the same volume of chloroform to extract the atrazine as mentioned above was repeated two times. Gathering the 30 mL chloroform was condensed into 1-mL volume by a rotary evaporator at 40 °C, and the atrazine concentration was determined. The atrazine concentration dissolved in the chloroform was determined by gas chromatography (GC-14C, Shimadzu, Japan). The detailed gas chromatographic condition and determining method were the same as previously reported (Zhang et al. 2011).

### Analysis of soil bacterial diversity

The total genomic DNA of the soil samples was extracted by Soil DNA Kit D-5625-01 of OMEGA. The PCR conditions are as follows: denaturation for 10 min at 94 °C, followed by

30 cycles of 94 °C for 30 s, 53 °C (anneal temperatures) for 20 s, 72 °C for 30 s, and a final extension at 72 °C for 10 min. Of the PCR product obtained in the above reaction, 20 μL was loaded onto 40 % (*w*/*v*) polyacrylamide gels in 50× TAE buffer. The polyacrylamide gels were made with a linear denaturing gradient ranging from 30 % denaturant at the top of the gel to 60 % denaturant at the bottom (100 % denaturant is defined as 7 M urea and 40 % (*v*/*v*) deionized formamide). The electrophoresis was run at 180 V and 60 °C for 300 min. Gels were stained with ethidium bromide for 20 min, rinsed with distillate water, and photographed with U.V. illumination in the Fluor-S Multi Molecular Imager (Bio-Rad, USA) to obtain a DGGE band image. Then, DGGE figure was analyzed for microbial diversity using BandScan and Quantity One analyses software applications.

### Biological test of the bioremediation effects

In order to evaluate the effect of the remediated soil on agricultural crops, besides the four treatments described above, another two treatments were added in this experiment as follows: unpolluted soil samples (treatment 5) and the soil samples without repair process (treatment 6). Ten soybean seeds were sown into the soils, and the soil moisture content was maintained at 25 %. Periodically, 50 mL water was added to the soil through the experimental period to maintain the moisture content. All the samples were cultured for 20 days. All soil treatments in this experiment were repeated in three replicates. Parameters measured during the period of the experiment are the plant height, plant weight, root length, and root weight of the soybean seedlings grown in differently treated soil samples. Each index mentioned above was measured three times, and the mean values were used to indicate the bioremediation effects.

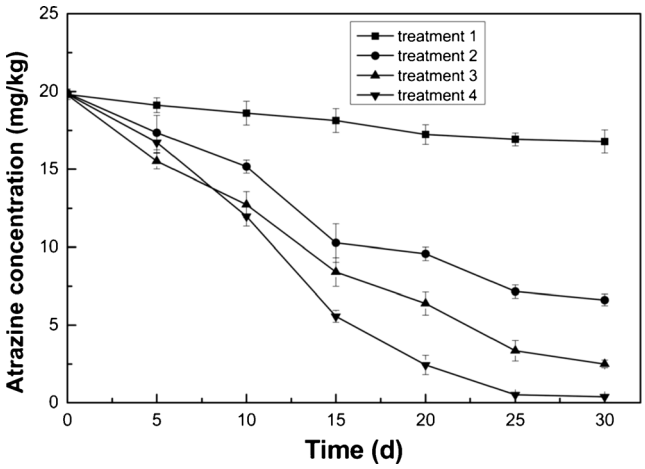


**Fig. 1** Bioremediation effect of atrazine-contaminated soil. The atrazine concentrations were measured at 5-day interval during the experimental period. *Errors bars* represent the mean (±S.D.) of three independent samples

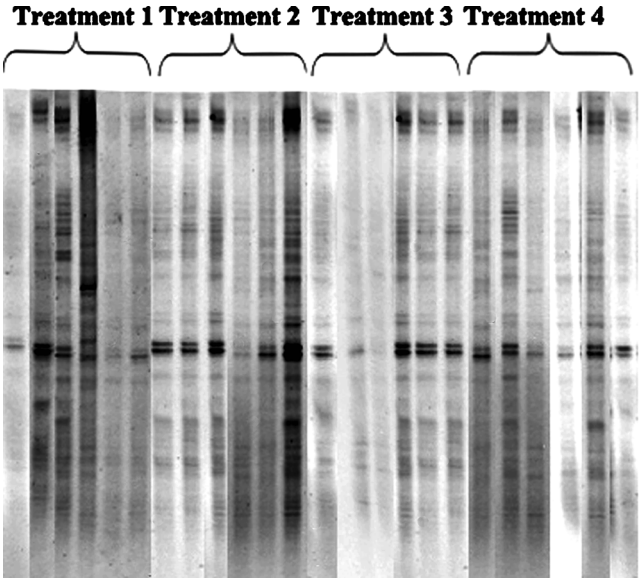


**Fig. 2** The results of soil bacterial diversity by DGGE gel during the bioremediation process. Every stripe in treatment 1, treatment 2, treatment 3, and treatment 4 represented the results DGGE of soil which was sampled at 5, 10, 15, 20, 25, and 30 days from *left* to *right*

## Results

### Bioremediation effect of atrazine-contaminated soil

Information about the change of atrazine concentration in different treatments is shown in Fig. 1. The initial concentration of atrazine used in the experiment was 19.52 mg $kg^{-1}$. During the experimental period, a slight decline was only observed in the concentration of atrazine in the soil of treatment 1. Contrarily, the atrazine concentrations of other three treatments (treatments 2, 3, and 4) changed significantly. These results indicated that *Pennisetum* and strain DNS10 used in this experiment altered the concentration of atrazine in the experimental units. In addition, treatments 2 to 4 exhibited different atrazine degrading rates. At the initial stage of the experiment (5 days), atrazine concentration in these three experimental units was observed to vary. With the culture time, the differences of atrazine concentrations between the

**Table 1** Microbial diversity of different treatments is analyzed by BandScan over the study period

| Time (days) | Treatment 1 | Treatment 2 | Treatment 3 | Treatment 4 |
|---|---|---|---|---|
| 5 | 19[a] | 20 | 20 | 20 |
| 10 | 18 | 21 | 23 | 24 |
| 15 | 17 | 25 | 27 | 31 |
| 20 | 15 | 23 | 24 | 30 |
| 25 | 16 | 22 | 21 | 28 |
| 30 | 15 | 21 | 21 | 25 |

[a] Represents the amount of species analyzed by BandScan

**Table 2** The Shannon–Weiner index of different treatments is analyzed by Quantity One over the study period

| Time (days) | Treatment 1 | Treatment 2 | Treatment 3 | Treatment 4 |
|---|---|---|---|---|
| 5 | 2.35[a] | 2.34 | 2.35 | 2.39 |
| 10 | 2.30 | 2.39 | 2.46 | 2.45 |
| 15 | 2.30 | 2.49 | 2.54 | 2.64 |
| 20 | 2.25 | 2.38 | 2.49 | 2.63 |
| 25 | 2.23 | 2.38 | 2.47 | 2.59 |
| 30 | 2.20 | 2.33 | 2.45 | 2.53 |

[a] Represents the Shannon–Weiner index

three treatments (treatment 2–4) were significant. After 20 days, atrazine degradation rates of these three treatments were 51.90, 67.70, and 87.77 %, respectively. At the end of the culture (30 days) time, atrazine degradation rates were 66.71, 87.38, and 98.10 %, respectively. Soil samples of the treatment with combined plant–microbe interactions (treatment 4) had no atrazine detected at the end of the experimental period.

### Concentration detected at the end of the experimental period

This research utilized PCR–DGGE techniques for the investigation of the changes of soil bacterial diversity during the experimental period. The soil bacterial diversity was determined by analyzing the results of DGGE gel according to BandScan and Quantity One. The results of DGGE gel are presented in Fig. 2, and the Shannon–Weiner index of the soil samples is shown in Tables 1 and 2.

The results in Table 1 show that within the early stage of the experiment (0 to 5 days), the rate of atrazine degradation among the treatments was insignificant. But after 25 days of the experiment, an increase in species number of treatment 4 beyond that of the other treatments was observed. On the contrary, a decline in the microbial diversity of treatment 1 was observed. The number of species in the other treatments significantly increased during 10–15 days and reached the highest at the 15 days. The number of species in other three treatments (2, 3, and 4) attained 25, 27, and 31, respectively. In addition, the Shannon–Weiner index of treatment 1 compared to other treatments was the least, and the highest Shannon–Weiner index was 2.35. Contrarily, treatment 4 had the higher Shannon–Weiner index than other treatments, since it reached the highest Shannon–Weiner index of 2.64 at 15 days.

### Biological test result of bioremediation effects

The growth characteristics of soybean with different treatments mentioned above are shown in Table 3. The best performing soybean plants were those grown in soil amended with treatment 5, while the least performing plants were those obtained from treatment 6. In addition, no significant ($P < 0.05$, $n = 3$) difference was observed between the plant height of soybean seedling in treatment 3 and treatment 4. They were second only to soybean seedling's growth in treatment 6 on this index and their heights were 31.98 and 31.76 cm, respectively. Furthermore, growth of soybean seedlings of treatment 3 was higher compared with that of treatment 4 on the basis of their plant and root weights but lower on the basis of the plant length. In all the treatments, atrazine concentration of treatment 5 (without repair process soil) was none, yet that of treatment 6 (unpolluted soil samples) was the highest. It was observed that the growth of soybean reduced significantly ($P < 0.05$, $n = 3$) with exposure to increasing concentrations of atrazine.

## Discussions

The study results on bioremediation effect of atrazine-contaminated soil describe the potential of atrazine degradation with the combination of the plant and microbial inoculation. Application of atrazine-degrading microbes which played an important role could significantly enhance the degradation of atrazine residues in soil. The rate of atrazine degradation by treatment 4 was faster than other treatments. It might due to the microbial activity of strain DNS10 and degradation mediated from plant exudates in root zone (Singh et al. 2004). Meanwhile, this result also might infer that plant roots could help in the spread of bacteria through soil, hence penetrating into deeper soil layers through the dissimilar root morphologies (Kirk et al. 2005; Lai et al. 2009). In addition, atrazine could be degraded to cyanuric acid by strain DNS10

**Table 3** Growth characteristics of soybean after a 3-week exposure to soil which was repaired by pot experiment (treatments 1–4), uncontaminated (treatment 5), and not repaired (treatment 6)

[a] Represents the mean (±S.D.) of the growth characteristics of three independent individual soybeans after a 3-week exposure to soil

| | Plant height (cm) | Plant weight (g) | Root length (cm) | Root weight (g) |
|---|---|---|---|---|
| Treatment1 | 28.29±1.75c[a] | 1.5622±0.14c | 13.23±1.56c | 0.4724±0.03d |
| Treatment 2 | 30.89±1.12b | 1.8881±0.19b | 17.43±2.67b | 0.8534±0.07c |
| Treatment 3 | 31.98±2.66ab | 2.2588±0.17a | 18.22±1.67b | 1.0575±0.13ab |
| Treatment4 | 31.76±1.47ab | 2.0761±0.37ab | 19.61±1.40ab | 0.9234±0.17bc |
| Treatment 5 | 33.83±0.82a | 2.3092±0.26a | 20.44±0.62a | 1.1962±0.21a |
| Treatment 6 | 24.96±3.10d | 1.3201±0.08c | 10.45±0.98d | 0.3327±0.04d |

(Zhang et al. 2011). And then, urea, a nitrogen fertilizer, could be produced from cyanuric acid by the soil microorganism to promote the growth of plant (El-Sayed et al. 2006; Cheng et al. 2005). All these hypotheses suppose a synergistic effect between plants and microorganisms.

The results above indicated that the bacterial diversity levels of the soils were raised in the process of plant–microbial combined bioremediation. The functional diversity of soil microbial communities was correlated with aboveground plant biomass and root biomass (Liu et al. 2010). The growth of the plant *Pennisetum* was affected by other environmental factors such as temperature, pH, nutritional status, oxygen, water, and other biologic factors, which similarly contributed to the degradation process of the atrazine. Therefore, the treatments which were added with *Pennisetum* (treatment 2 and treatment 4) showed a higher microbial diversity and *Pennisetum* played an important role in promoting the bacterial activity. It was the viewpoint that the main mechanism of atrazine degradation in soil was a result of the activity of the indigenous and inoculated microorganisms. It could be inferred that *Pennisetum* created the feasible growing conditions for the microorganisms and promoted the number and the activity of the soil microorganisms. Consequently, the atrazine degradation effect of treatment 4 was better compared with other treatments.

Most of the current reports evaluated the repair effect based on the rate of atrazine reduction. However, in nature, sensitive plants may have the potential to produce significant physiological changes to atrazine at low concentrations. Thus, it was more suggestive to use sensitive plants to further test the soil after remediation with treatment 4 having the best growing conditions. From another perspective, this study introduced a basic growth index for soybean seedlings grown in soils with different treatment applications. Based on this hypothesis, the effect of atrazine concentration in the soil on the plant was monitored. Soybean seedlings grown in the soils remediated with the application of the plant–microbe interaction system had a significant effect on repairing soils contaminated by atrazine compared with the lone application of either of the treatments, given the same repairing time and conditions. And the soybean seedlings in the soils of treatment 4 also revealed that the resistant plant and the bacterial strain DNS10 did not affect the sustainable utilization of soils but had minor side effects on the environment when they repair soils contaminated by atrazine.

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