Isolation of two atrazine-degrading strains and their degradation characteristics

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Abstract: To investigate the optimum growth conditions and screen atrazine-degrading strains, two atrazine-degrading strains named Z_9 and Z_{42} were isolated from black earth in a cold area with a long-term application of atrazine by standard enrichment techniques. Z_9 utilizes atrazine as both the nitrogen and carbon source whereas Z_{42} utilizes atrazine as the sole nitrogen source to grow. The atrazine degradation rates of the two strains reached 77.7% and 65.6%, respectively after 14 days culture in a liquid medium with an atrazine concentration of 100 mg/L. Z_9 and Z_{42} were identified as *Microbacterium sp.* and *Arthrobacter sp.* The optimum inoculation amount and rotation speed for Z_9 and Z_{42} to grow and degrade atrazine are 3% and 120 r/min respectively.

Keywords: atrazine, isolation, identification, degradation characteristics

DOI: 10.3965/j.issn.1934-6344.2009.03.027-032

Citation: Ying Zhang, Zhou Ning, Jiang Zhao, Peng Xinran, Ma Shuyan, Hu Miao. Isolation of two atrazine-degrading strains and their degradation characteristics. Int J Agric & Biol Eng, 2009; 2(3): 27－32.

1 Introduction

Atrazine is a kind of selective inner-absorption conductive herbicide. It can be applied to corn, sorghum, sugar cane, tea gardens, orchards, Korean pine seedling nursery, woodland to kill both annual and broadleaf weeds. It can also damage some perennial weeds. Atrazine is an important herbicide in agricultural production and can bring about enormous economic benefits. It has been recognized as an excellent herbicide for a long time. At the same time, it also causes pollution to the environment and bring great harm to many kinds of organism[1-3]. For example, excessive use of atrazine would accumulate residue level of it in the soil and would affect the growth of succeeding crops, especially legumes[4]. Atrazine residue in the soil and sprayed to cultivate the agricultural products can enter into the groundwater and surface water during irrigation of farmland[5,6]. Therefore, soil, surface water, groundwater and atmosphere could be seriously polluted by atrazine[7,8]. Atrazine can also affect human and other animals directly because it can be concentrated by plants and transferred to the food chain and its biological toxicity[7].

Many studies have shown that microorganisms in water and soil have the ability to degrade atrazine. Therefore, it is important to isolate strains with high degradation efficiency. Some countries have been committed to isolate the atrazine-degrading strain since 1960s. Presently, the isolated atrazine-degrading strains include bacteria, fungi, algae and actinomycetes such as *Rhodococcus*[8], *Pseudomonas*[9,10], *Agrobacterium*[9], *Acinetobacter*[11], *Rhizobium*[12], *Rhizopus*, *Aspergllus*, *Aspergillususstus*, *Fusarium*, *Penicillium*, *Trichoolerma*, *White rot fungi* and so on[13,14]. Because of the diversity of biochemical characteristics and the strong ability to

Received date: 2009-03-16 **Accepted date:** 2009-07-28

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adapt to the environment, the bacteria plays an important role in atrazine degradation.

Microorganisms from nature or special cultivation can transform toxic pollutants into innocuous substance through their metabolism process and reduce or even eliminate toxicity of environmental pollutants so as to decrease the harms to human health and the ecosystem caused by atrazine. Therefore, many countries have paid great attention to studies of bioremediation of atrazine by microbes.

In this paper, two atrazine-degrading strains were isolated from the soil of Heilongjiang Province of China and identified by physiological and biochemical tests.

The optimum growth and degradation conditions of the two strains in liquid medium with an atrazine concentration of 100 mg/L were also investigated.

2 Materials and methods

2.1 Soil samples

The soil used was collected from topsoil (0–10 cm) of a cornfield in Heilongjiang Province of China with long-term atrazine application. One kilogram of soil was sampled from five positions in this site and mixed evenly.

2.2 Herbicide (Atrazine)

The purity of atrazine was 97% and was produced by Wing Int'l Trading Co., Ltd, Wuxi, Jiangsu Province, China.

2.3 Medium

2.3.1 Sole carbon source medium

K_2PO_4 1.6 g, KH_2PO_4 0.4 g, $MgSO_4$ 0.2 g, NaCl 0.1 g, NH_4NO_3 0.5 g, trace elements solution 1 mL, suitable amounts of atrazine as the sole carbon source, distillation water 1000 mL, 121℃ sterilization for 30 min.

2.3.2 Sole nitrogen source medium

K_2PO_4 1.6 g, KH_2PO_4 0.4 g, $MgSO_4$ 0.2 g, NaCl 0.1 g, sucrose 3.0 g, trace elements solution 1 mL, suitable amounts of atrazine as the sole nitrogen source, distillation water 1000 mL, 121℃ sterilization for 30 min.

2.3.3 Sole carbon and nitrogen source medium

K_2PO_4 1.6 g, KH_2PO_4 0.4 g, $MgSO_4$ 0.2 g, NaCl 0.1 g, trace elements solution 1 mL, suitable amounts of atrazine as sole carbon and nitrogen source, distillation water 1000 mL, 121℃ sterilization for 30 min.

2.4 Isolation of atrazine-degrading strains

Ten-grams of soil was added into 90 mL culture medium with an atrazine concentration of 100 mg/L and cultured at 30℃ in a rotary shaker (150 r/min) for one week. 10 mL of this solution was added into 90 mL of fresh culture medium and the concentration of atrazine in the cultural medium was increased gradually. Repeat the transfer process said above every week. After two months enrichment, the liquid medium was plated on solid culture medium and cultured at 30 ℃. Morphologically distinct colonies were selected for plate clearance assay.

2.5 Extraction of atrazine

The atrazine in the medium was repartitioned with dichloromethane whose volume was the same as the medium after 5 min shaking. The dichloromethane fractions were pooled, passed through anhydrous sodium sulfate and concentrated to near dryness in a rotary evaporator below 45℃ under reduced pressure. Fixed the volume of the extracts was 1 mL with dichloromethane before determination.

2.6 Determination of atrazine

The extracted atrazine was analyzed by a Shimadzu gas chromatograph (GC-14C) equipped with a fused silica chromatographic column (300 mm×0.53 mm) packed with 14% OV-1701 and a flame ionization detector (FID) using N_2 as the carrier gas. The injection and FID detector temperatures were 250℃ and the column temperature was 200℃, respectively.

2.7 Calculation of atrazine biodegradation rate

The atrazine degradation rate was calculated by the following formula:

$$X\% = \frac{C_{ck} - C_x}{C_{ck}} \times 100\%$$

where, X is atrazine degradation rate; Cx is the concentration of atrazine (mg/L) in the medium that has atrazine degrading strain; C_{CK} is the concentration of atrazine (mg/L) in the medium that does not contain atrazine degrading strain.

2.8 Identification of atrazine-degrading strains

Preliminary identification of the isolated strains was

performed by routine morphological and physiological and biochemical tests in accordance with the "common bacterial identification manual system" [15].

2.9 Growing and degrading characteristics of Z_9 and Z_{42} under different cultural conditions

2.9.1 Atrazine concentration

The atrazine concentrations in the liquid mediums were 10, 50, 100, 200, 500 mg/L. Inoculating Z_9 and Z_{42} into the fresh medium mentioned above by adding Z_9 and Z_{42} solution(OD_{600}=1) by the ratio of 1%(V/V). All the samples were cultured at 30℃ and 150 r/min. The OD_{600} of the medium which represent the growth of strain and the atrazine concentration of the mediums were recorded.

2.9.2 Inoculation amount

The fresh liquid medium was prepared with the same composition as the medium used for the culture of Z_9 and Z_{42}. The atrazine concentration of these mediums was 100 mg/L. Inoculating Z_9 and Z_{42} solution whose OD_{600} was 1 into the fresh medium with a ratio of 1%, 2%, 3%, 4% and 5%(V/V), respectively. All the samples were cultured at 30℃ and 150 r/min. Detected the OD_{600} and of the atrazine concentration medium.

2.9.3 Rotation speed of shaking table

Some amount of fresh liquid medium was prepared as said in 2.9.2. The atrazine concentration of these medium was 100 mg/L. Z_9 and Z_{42} solution whose OD_{600} is 1 was inoculated into the fresh medium by the ratio of 1%. All the samples were cultured at 30℃ and 0, 40, 80, 120, 160, and 200 r/min, respectively. The OD_{600} and the atrazine concentration of the mediums were recorded.

3 Results and discussion

3.1 Sieving of atrazine-degrading strains

Fifty-nine bacterial strains were isolated from the collected soil samples and their degrading abilities were determined. There are eight bacterial strains that can degrade atrazine. Z_9 and Z_{42} can utilize atrazine as the sole carbon, nitrogen source and sole carbon source to grow. The atrazine-degrading rates of Z_9 and Z_{42} after 14 days culture were 77.7% and 65.6% respectively and better than that of other strains. Therefore, Z_9 and Z_{42} were chosen as the selected strains for the further study.

3.2 Identification of atrazine-degrading strains

The colony of Z_9 is larger. The surface of colony is concave and the color was opaque and bright. It has anomalous edge. Gram stain indicated this bacterium is gram-positive. The picture of scanning electron micrograph (SEM) shows that the strain had a short rod shape and its size is about 0.99−1.23 μm×0.56−0.59 μm.

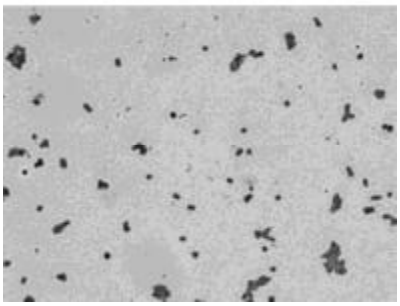

Figure 1 Gram picture of Z_9 (10×100)

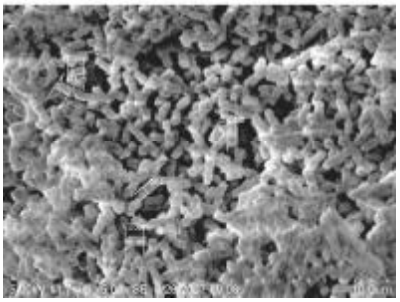

Figure 2 Scanning electron micrograph of Z_9 (5000×)

The colony of Z_{42} is smaller and has no anomalous edge. It has a convex surface. The color is opaque, bright and ivory white. Gram stain indicated this bacterium is gram-positive. Z_{42} has a short rod cell shape and its size is about 1.21−1.14 μm× 0.40−0.51 μm observed under SEM.

From the configuration of the strains and the physiological and biochemical test results, Z_9 and Z_{42} are identified to be *Microbacterium sp* and *Arthrobacter sp* primarily basing on "common bacterial identification manual system".

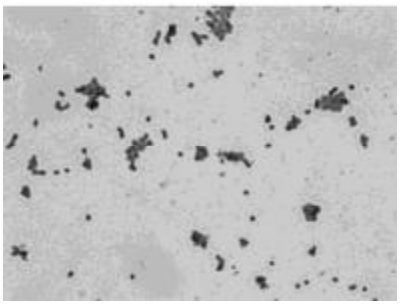

Figure 3 Gram picture of Z_{42} (10×100)

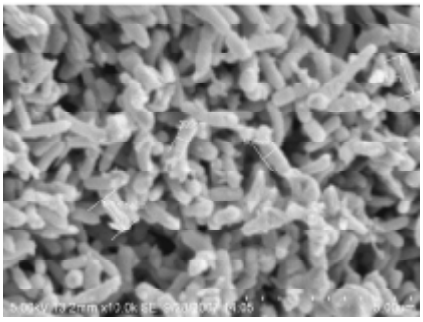

Figure 4 Scanning electron micrograph of Z_{42} (10000×)

3.3 Growing and degrading characteristics of Z_9 and Z_{42} at different cultural conditions

3.3.1 Atrazine concentration

The growth and degradation characteristics of Z_9 and Z_{42} in the medium with different atrazine concentrations are shown in Figures 5 and 6.

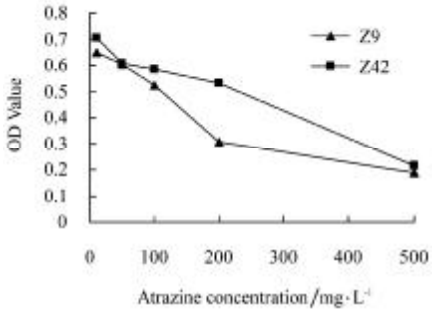

Figure 5 Effects of atrazine concentration on the growth of bacteria

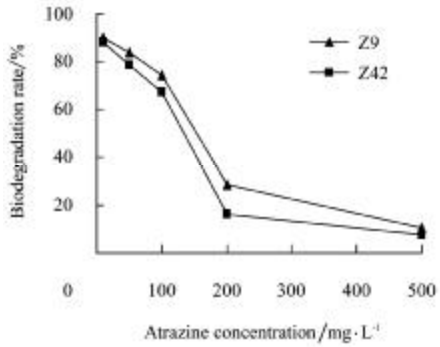

Figure 6 Effects of atrazine concentration on the atrazine biodegradation rate

Figures 5 and 6 indicate that the growth of the two strains was greatly affected by the concentration of atrazine and the high concentration of atrazine was harmful to the growth and degradation abilities of Z_9 and Z_{42}. When the concentration of atrazine changed from 10 mg/L to 500 mg/L, the OD_{600} and the degradation rate of the two strains decreased gradually. The reason may be that the atrazine is a complex compound and has biological toxicity, so it can cause poisoning to strain and the growth of strains decreases when the atrazine concentration was increased.

The two figures also show that there exists a suitable atrazine concentration for a certain amount of strains to degrade it. At this concentration the growing amount and degradation rate of strains were relatively higher than any other times. Therefore, the relationship between the quantity of strains and the concentration of herbicide should be given full consideration in actual application. Analyzing the degradation rate and growth condition of Z_9 and Z_{42} in media with different atrazine concentrations, shows that the two strains still have the excellent degradation and growth abilities in the medium whose atrazine concentration is 100 mg/L. So, this atrazine concentration needs further research.

3.3.2 Inoculation amount

The growth and degradation characteristics of Z_9 and Z_{42} in the condition of different inoculation amount are shown in Figures 7 and 8.

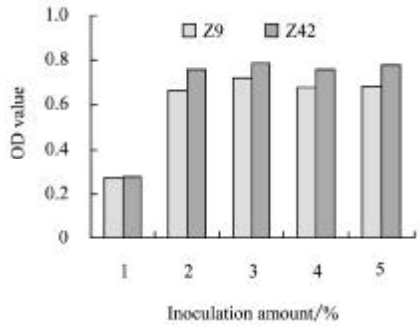

Figure 7 Effects of inoculation amount on the growth of bacteria

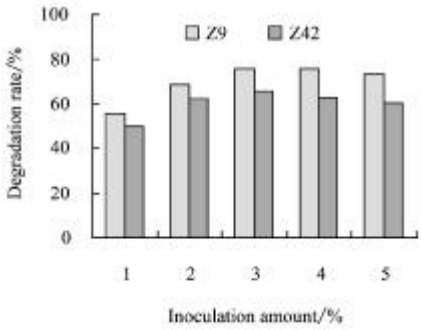

Figure 8 Effects of inoculation amount on the atrazine degradation rate

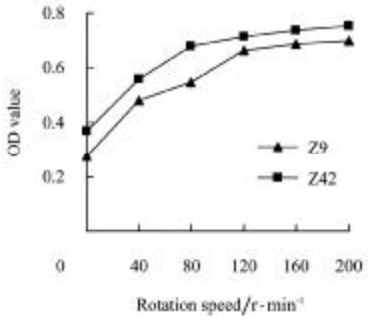

Figure 9 Effects of rotation speed on the growth of bacteria

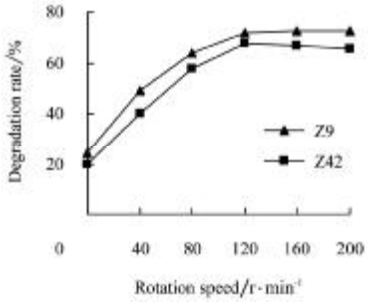

Figure 10 Effects of rotation speed on the atrazine degradation rate

Figure 7 shows that when the inoculation amount of bacterial suspension increased from 2% to 5%, Z_9 and Z_{42} grew well. However, when inoculation amount reached 3%, OD_{600} values of Z_9 and Z_{42} were up to 0.722 and 0.786, respectively. Figure 8 indicates that the initial inoculation amount has significant impact on the atrazine degradation.

In this study, the degradation rate of Z_9 and Z_{42} reached 75.8% and 65.5% respectively when the inoculation amount was 3%. The reason for this phenomenon may be that: when the inoculation amount was less, the strains were not in sufficient contact with the atrazine in medium, so the degradation rate was lower; thus, when the inoculation amount was more, carbon source competition would occur in initial stages of the strains growth which resulted in strains malnutrition and having significant influence on biodegradation rate accordingly.

3.3.3 Rotation speed of shaking table

The growth and degradation characteristics of Z_9 and Z_{42} cultured at different rotation speed are shown in Figures 9 and 10.

Figure 9 shows that the higher rotation speed is, the more favorable for atrazine-degrading strains to grow. In certain range, increasing the rotation speed could increase the dissolved oxygen in the culture mediums and improve uniformity of mixing between atrazine and strains. This indicated that both of the two strains are aerobic bacteria.

Figure 10 indicates that different rotation speeds of the shaking table has different influence on atrazine degradation rate of the two strains. It shows that when the rotation speed is less than 120 r/min, the degradation rate increases with the increase of rotating speed. The rotation speed may have an effect on the degradation rate indirectly through influencing the amount of dissolved oxygen in the culture medium. However, when the rotation speed was more than 120 r/min, the degradation rate did not change very much. This may because that the certain volume culture medium had certain amount of dissolved oxygen so that the degradation rate changed very little and even decreased slightly when the rotation speed was so high. Perhaps the bacterial cells were subjected to shear force which might decrease their

ability to degrade atrazine. So the optimum rotation speed for Z_9 and Z_{42} to grow and degrade atrazine was 120 r/min.

4 Conclusions

Two atrazine–degrading strains named Z_9 and Z_{42} were isolated from topsoil of Heilongjiang Province by standard enrichment techniques. Both of them can utilize atrazine as nourishment to grow and degrade atrazine accordingly. The degradation rates of Z_9 and Z_{42} can reach 77.7% and 65.6% respectively after 14 days' culture;

Z_9 utilized atrazine as the sole nitrogen and carbon source to grow and atrazine was the nitrogen source to sustain Z_{42} to grow. Z_9 and Z_{42} were preliminarily identified as *Microbacterium sp* and *Arthrobacter sp* according to the configuration observing and biochemical testing;

The optimum inoculation amount and rotation speed for Z_9 and Z_{42} to grow and degrade atrazine in the medium containing 100 mg/L atrazine are 3% and 120 r/min.

Acknowledgment

This research was supported by National Natural Science Foundation（30970525）, Key Science and Technology Project of China-Israel Cooperation Fund of Department of Science and Technology, Heilongjiang Province, China (WB07N01); National Scientific and Technological Supporting Project, China (2007BAD87B03); Natural Science Fund Project of Heilongjiang Province (E2007-16); Main Youth Fund of Department of Education of Heilongjiang Province (1152G006); Innovating Group Fund of Northeast Agricultural University(CXT003-1-2).

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