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Biodegradation of Crystalline and Nonaqueous Phase Liquid-Dissolved ATRAZINE by *Arthrobacter* sp. ST11 with Cd^{2+} Resistance

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Abstract: A newly isolated cadmium (Cd)-resistant bacterial strain from herbicides-polluted soil in China could use atrazine as the sole carbon, nitrogen, and energy source for growth in a mineral salt medium (MSM). Based on 16S rRNA gene sequence analysis and physiochemical tests, the bacterium was identified as *Arthrobacter* sp. and named ST11. The biodegradation of atrazine by ST11 was investigated in experiments, with the compound present either as crystals or dissolved in di(2-ethylhexyl) phthalate (DEHP) as a non-aqueous phase liquid (NAPL). After 48 h, ST11 consumed 68% of the crystalline atrazine in MSM. After being dissolved in DEHP, the degradation ratio of atrazine was reduced to 55% under the same conditions. Obviously, the NAPL-dissolved atrazine has lower bioavailability than the crystalline atrazine. Cd^{2+} at concentrations of 0.05–1.5 mmol/L either had no effect (<0.3 mmol/L), slight effects (0.5–1.0 mmol/L), or significantly (1.5 mmol/L) inhibited the growth of ST11 in Luria-Bertani medium. Correspondingly, in the whole concentration range (0.05–1.5 mmol/L), Cd^{2+} promoted ST11 to degrade atrazine, whether crystalline or dissolved in DEHP. Refusal to adsorb Cd^{2+} may be the main mechanism of high Cd resistance in ST11 cells. These results may provide valuable insights for the microbial treatment of arable soil co-polluted by atrazine and Cd.

Keywords: atrazine; *Arthrobacter* sp. ST11; biodegradation; nonaqueous-phase liquid; cadmium

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1. Introduction

Arable soil is often polluted with herbicides [1]. Atrazine (6-chloro-4-N-ethyl-2-N-propan-2-yl-1,3,5-triazine-2,4-diamine) is one of the most widely-used persistent chlorine herbicides that often remain in agricultural fields and water bodies for several years at concentrations of hundreds of μg/kg [2]. Atrazine has lower bio-accessibility when present in an unavailable phase, such as crystalline form, soil and sediment solids, or non-aqueous phase liquid (NAPL). The environmental persistence of atrazine has been shown to be a vastly significant problem [2]. No strong evidence could prove that atrazine causes cancer; however, it affects the endocrine response and thus has a potential effect on human reproduction and development [3]. Although it was banned for use by the European Union in 2004, atrazine remains legal in China [2]. Atrazine is expected to persist in arable soil sources for decades [4], thereby calling for appropriate remediation measures.

Cadmium (Cd), a potentially toxic heavy metal with no known biological function, occurs widely in nature [5]. A national-scale study of soil Cd pollution in China reported that the average and maximum concentrations of Cd in arable soil were 0.0024 and 1.36 mmol/kg, respectively [6]. As one of the most toxic trace elements in the environment, Cd could cause serious health problems to microorganisms, plants, animals, and humans [5,7].

Bioremediation is cost-effective and environmentally friendly and, thus, has become one of the most popular approaches for removing atrazine [2]. Biodegradation by indigenous

microbial populations is considered an important process that affects the fate of atrazine in contaminated sites [2]. Since the 1990s, different atrazine-degrading bacteria and fungi have been isolated from contaminated sites [8,9], including *Arthrobacter* [10], *Nocardioides* [11], *Shewanella* [12], *Rhodococcus* [13], *Stenotrophomonas* [14], *Pseudomonas* [15], *Paenarthrobacter* [16], and *Trametes* [17]. Bacteria are the foundation of microbial bioremediation, which can outperform fungi in the potential for atrazine-specific bioaugmentation [18]. However, the low bioavailability of atrazine in the environment hindered the degradation efficiency of these strains. Although many enhancement methods have been applied [19–21], little is known about the mode of acquisition of atrazine when it is present in an unavailable phase. Heavy metals and synthetic pesticides often co-occur in soil, although their hazards are usually evaluated separately and in bulk soil [22]. Coexisting heavy metals may stimulate or inhibit the biodegradation of herbicides [23,24]. When Cd pollution coexists, the fate of atrazine in the soil will be difficult to predict. The potential ecological risk of combined pollution of atrazine and Cd in waters and soils still exists, and it cannot be ignored, even when said risk is lower than that of atrazine or Cd alone [25,26]. Overall, the search and isolation of specific bacterial strains that could degrade atrazine efficiently with Cd resistance are of great interest.

Arthrobacter is prevalent in the agricultural soil environment, and it could degrade many kinds of environmental pollutants [10,27]. Many *Arthrobacter* strains have been isolated to degrade atrazine, such as *Arthrobacter* Sp. DNS10, *Arthrobacter* sp. LY-1, and *Arthrobacter Aurescens* TC1 [28–30]. In addition, some *Arthrobacter* strains were reported to have metal resistance [31,32]. However, atrazine-degrading *Arthrobacter* strains with Cd resistance have not yet been found.

The three primary goals of this study were designed to address gaps that currently exist in the research. The first goal was to isolate high-efficiency atrazine-degrading bacteria with Cd resistance and characterize them through 16S rRNA gene sequencing analysis and physiochemical tests. The second goal was to investigate the bioavailability of crystalline and NAPL-dissolved atrazine to the strain. The third goal was to explore the mechanisms of the effect of Cd^{2+} on atrazine biodegradation. Finally, some valuable insights into the treatment of atrazine in soil with Cd pollution were provided.

2. Results and Discussion

2.1. Identification and Characterization of Test Strain

An efficient atrazine-degrading bacterium ST11 was isolated from herbicide-polluted soil. Under scanning electron microscopy (SEM), ST11 appeared as rods when rapidly dividing and cocci when in the stationary phase (Figure S1), showing the typical characteristics of *Arthrobacter* cells [33]. The strain was further identified as *Arthrobacter* sp. by physiochemical tests (Table S1) and 16S rDNA analysis (GenBank OP435654). A phylogenetic tree was constructed using an approximate maximum-likelihood analysis (Figure 1).

2.2. Characterization of Cd^{2+} Resistance

The ST11 strain was assessed for its ability to grow in the presence of Cd^{2+}. As shown in Figure 2, no difference was found from the control samples when ST11 was incubated for 48 h in a culture medium containing <0.3 mmol/L Cd^{2+}. The growth of ST11 was slightly suppressed in the presence of 0.5–1.0 mmol/L Cd^{2+} and significantly suppressed in the presence of 1.5 mmol/L Cd^{2+}. The effective concentration-25 (EC_{25}) and effective concentration-50 (EC_{50}) were defined as the Cd^{2+} concentration required to obtain 25% and 50% cell growth inhibition effects, respectively. The EC_{25} and EC_{50} of ST11 toward Cd^{2+} were 1.21 and 2.01 mmol/L, respectively.

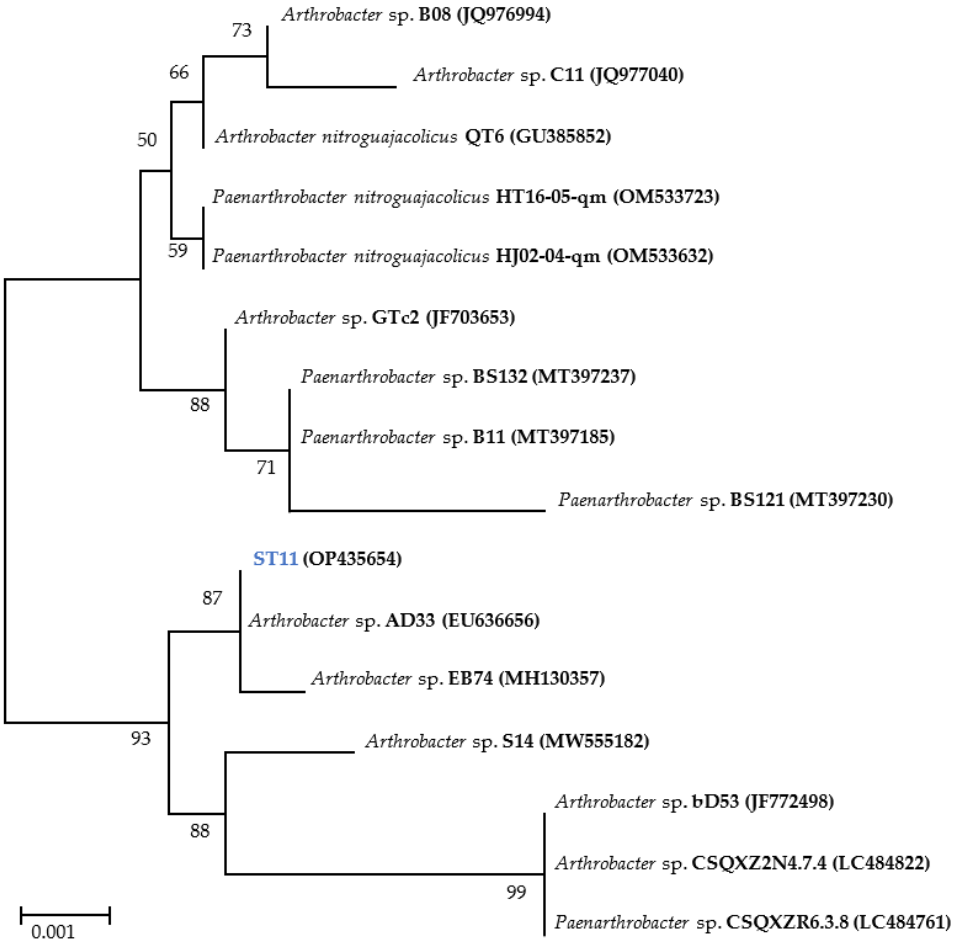

Figure 1. Phylogenetic analysis of strain ST11 and related species by the Neighbor-Joining method based on 16S rRNA gene sequences. Bootstrap values (%) are indicated at the nodes, and the scale bars represent 0.001 substitutions per site.

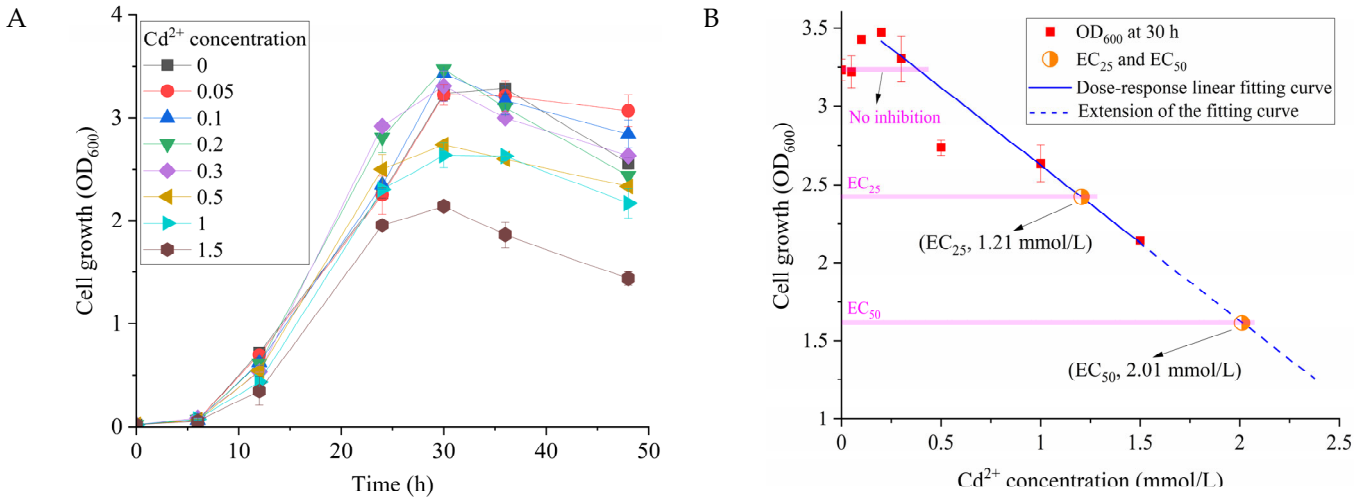

Figure 2. Growth curves (**A**) and inhibition fitting curve (**B**) of ST11 in the presence of Cd^{2+}. The cells were cultured with the Luria-Bertani (LB) medium in the presence of Cd^{2+} (0–1.5 mmol/L) at 30 °C at 150 rpm in darkness for 48 h. At the timed interval, flasks were taken out and 1 mL culture was withdrawn for OD_{600} measurements.

The adsorption capacities of ST11 for Cd^{2+} were shown in Figure 3. Before and after cell adsorption, the difference of Cd^{2+} detected was negligible. The result shows that ST11 cells have limit adsorption capacity for Cd^{2+}.

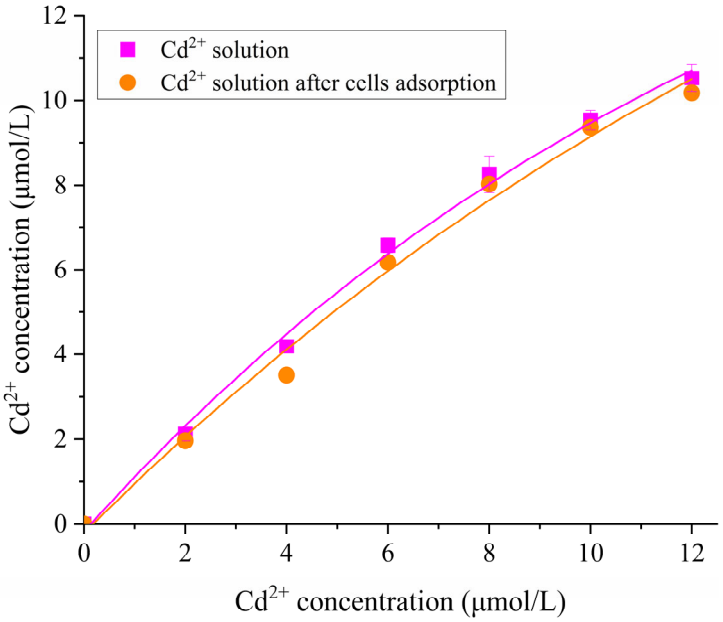

Figure 3. Adsorption isotherm of Cd^{2+} on ST11 cells as a function of Cd^{2+} concentration. The cells were harvested at 6380× *g* centrifugation for 10 min after ST11 was cultured in LB for 48 h. The cells were washed three times with deionized water to remove the residual medium. The obtained wet cells were used directly for Cd^{2+} adsorption without further treatment. The adsorption of Cd^{2+} by ST11 cells was carried out at 25 °C and pH 7.

Arthrobacter is prevalent in the agricultural soil environment, and it could degrade many kinds of environmental pollutants, but evidence of its resistance to heavy metals is limited [10]. For instance, a quinaldine-degrading *Arthrobacter* sp. Rue61a was suggested to have the potential to tolerate heavy metals, such as Zn^{2+}, Pb^{2+}, and Co^{2+} [31]. However, for atrazine-degrading *Arthrobacter*, a strain with the ability to resist heavy metals has not been reported. In this study, *Arthrobacter* sp. ST11 was shown to survive in the presence of up to 1.5 mmol/L Cd^{2+}. The microbial metabolism of heavy metals includes extracellular complexation, extracellular precipitation, cation outflow, in-vivo detoxification, and in vivo complexation [32]. Living ST11 cells do not adsorb Cd^{2+}, so the resistance of ST11 to heavy metals may be based on cation outflow or the absence of a Cd^{2+} binding site on the cell membrane of ST11.

2.3. Biodegradation of Crystalline and NAPL-Dissolved Atrazine

The cell growth and biodegradation of crystalline and NAPL-dissolved atrazine by ST11 are shown in Figure 4A,B, respectively. Di(2-ethylhexyl) phthalate (DEHP) is a commonly used typical NAPL [34] that is biocompatible and could not be used by ST11. Meanwhile, atrazine could be degraded by physical, chemical, and biological methods [35]. In the absence of microorganisms, they could attenuate naturally under some physical and chemical factors [18]. A non-inoculated control experiment was set up in the present study to exclude the effects of temperature, pH, dissolved oxygen, ionic strength, and other physical and chemical factors. For the whole culture cycle, significant bacterial growth of non-investigated samples was not detected under these conditions. For inoculated samples, during the first 16 h, the numbers of cells produced at the two systems were not statistically (Student's *t*-test) significant. The bacterial growth rates during this phase were 0.0171 ± 0.0013 and 0.0185 ± 0.0005 OD_{600}/h with 1.85 mmol/L crystalline and DEHP-dissolved atrazine, respectively. Subsequent bacterial growth led to an increased number

of cells until growth stopped after 40 h. At that time, the average final OD_{600} values of cells were 0.61 ± 0.01 and 0.44 ± 0.03 with crystalline and DEHP-dissolved atrazine, respectively. In the following 8 h, the cells almost stopped growing. Most of the bioavailable atrazine at that point was assumed to be consumed. Therefore, residual atrazine was detected after 48 h of culture (Figure 4B). Physical and chemical factors were found to degrade atrazine by no more than 15%. With crystalline atrazine as the substrate, the degradation ratio of atrazine increased significantly and reached 68% after 48 h. When atrazine was dissolved in DEHP, the degradation ratio of atrazine decreased to less than 55%, which is consistent with the low number of cells.

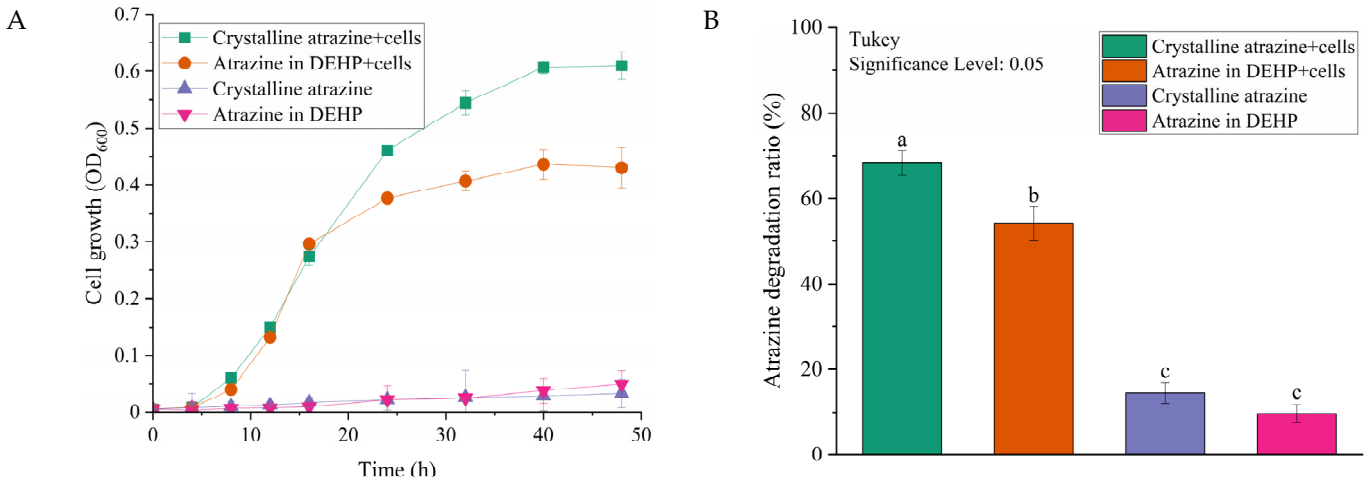

Figure 4. Cell growth (**A**) and biodegradation of atrazine (**B**) in crystalline form or in DEHP by ST11. The initial concentration of atrazine was 1.85 mmol/L. For biological samples, 1 mL of bacterial suspension (OD_{600} 1.0) was inoculated. For blank tests, no inoculation was prepared to control the non-biological effects. (**A**) The growth was monitored spectrophotometrically by measuring OD_{600} at an interval of 4–8 h. (**B**) At the end of culture (48 h), the biodegradation ratio of atrazine was detected. Data points represent the mean of three replicates and error bars show the standard deviation. Different lower-case letters (a, b, and c) over the bars indicate significant differences at $p < 0.05$.

The atrazine degradation pathway includes hydrolysis, deamination, dealkylation, and ring cleavage [2]. Liquid Chromatography-Ultraviolet (LC-UV) analysis was performed at 225 nm to determine whether a new metabolite was generated after atrazine degradation (Figure S2). A new absorption peak (Figure S2c) appeared at the retention time of 4.145 min, which was not found in the atrazine standard (Figure S2a) and the culture medium before atrazine was degraded (Figure S2b). In the process of atrazine biodegradation by the *Bacillus licheniformis* ATLJ-5 strain, intermediate metabolites hydroxyatrazine and n-isopropylammelide were detected by LC-UV [8]. Similarly, during the process of ST11 using atrazine, a UV detectable metabolite was found. This finding suggested that the new compound was a metabolite of the degraded atrazine.

Microscopic observation explained that atrazine dissolved in DEHP had decreased bioavailability (Figure 5). For the microbial adhesion to hydrocarbons (MATH) experiment of ST11, the solution was vigorously homogenized and then allowed to stand for 2 h to ensure complete phase separation. The OD_{600} of cells in the water phase increased from 0.6 to 0.68 (Figure 5B), possibly because some DEHP micro-droplets suspended in the water phase hindered the transmission of light. The DEHP oil phase (Figure 5B) was as clear and transparent as the sterile sample (Figure 5A), and no obvious emulsification was observed. The liquid at the oil-water interface was sampled for microscopic observation (Figure 5C). The results showed that the ST11 cells were evenly scattered in the water phase and not observed on the oil-water interface. Therefore, ST11 is a water-soluble bacterium that could only use atrazine dissolved in the water phase in the oil-water two-phase system.

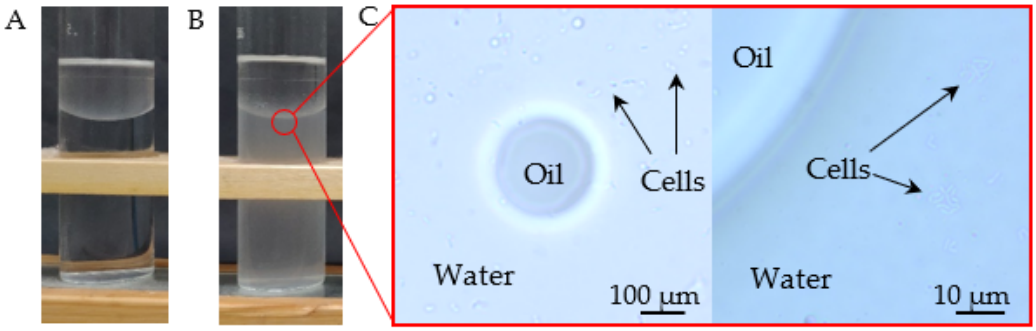

Figure 5. Visual aspect of two-phase systems with DEHP as a NAPL phase. (**A**) 5 mL of DEHP, 20 mL of MSM; (**B**) 5 mL of DEHP, 20 mL of cells suspension in MSM with OD_{600} 0.6; (**C**) Microscopic images of oil-water interface. The arrow indicates ST11 cells.

Atrazine has low bioavailability because of its low aqueous solubility (log P_{ow}, 2.28; water solubility, 0.153 mmol/L at 20 °C) [36]. However, when atrazine is used as a substrate in crystalline and DEHP-soluble forms, it could be speculated to have different bioavailability. The mass transfer of atrazine is not limited by the mass transfer rate, and ST11 cells could only use the substrate dissolved in water. When atrazine is a crystal, the mass transfer between the solid and water is only limited by the saturated solubility of atrazine. At this time, the concentration of atrazine in the aqueous phase could be approximately regarded as the saturated solubility (0.153 mmol/L). When atrazine is dissolved in a NALP, the mass transfer of atrazine between the NAPL and water is affected by the octanol-water partition coefficient (log P_{ow}, 2.28) of atrazine. In the experiments, the additional amount of atrazine in DEHP was 11.13 mmol/L. If the difference between DEHP and octanol was not considered, the concentration of atrazine in the aqueous phase was only 0.058 mmol/L. Therefore, when atrazine was used as a substrate in crystal form or dissolved in DEHP, the bioavailability of the former was 2.8 times that of the latter. The low bioavailability of atrazine in DEHP delayed its degradation by ST11 (Figure 4B).

For other hydrophobic organic compounds in soil, such as polycyclic aromatic hydrocarbons, the bioavailability mechanism of substrates in the non-aqueous phase has been deeply studied [34,37]. Although the bioremediation of atrazine has been extensively studied [38], little is known about the mode of its acquisition when it is present in an unavailable phase, such as soil and sediment solids or NAPL. For a naphthalene-degrading *Arthrobacter* sp., the attachment of this strain to the NAPL-water interface is necessary, and the cells at the interface could degrade organic compounds at rates higher than those of abiotic partitioning [39]. However, for ST11 cells that could not adhere to the oil-water interface (Figure 5), the biodegradation of atrazine in DEHP was limited by the mass transfer of the substrate between the two oil-water phases. Therefore, the findings of this study indicated that when atrazine is dissolved in NAPL in soil, its biodegradation is likely to be restricted, which may be one of the reasons why atrazine is a persistent contaminant in arable soil [4].

2.4. Effect of Cd^{2+} on the Growth of Strain ST11 and Atrazine Biodegradation

The effects of Cd^{2+} on ST11 cell density and degradation of atrazine in crystal form or dissolved in DEHP were determined (Figure 6). At Cd^{2+} concentrations below 0.3 mmol/L, cell growth and atrazine degradation were stimulated with Cd^{2+} concentration. The highest removal of atrazine (almost 100% at 48 h) in crystal form or dissolved in DEHP occurred in the case of Cd^{2+} concentration of 0.1 and 0.2 mmol/L, respectively. The DEHP-dissolved atrazine with decreased bioavailability, which may cause more Cd^{2+}, is needed to obtain high degradation ratio. When the concentration of Cd^{2+} was increased from 0.5 to 1.5 mmol/L, the effect of stimulating cell growth and atrazine degradation gradually weakened. However, in this Cd^{2+} concentration range, the growth was partially inhibited when ST11 was used soluble substrates (Figure 2A). A notable detail that at the same Cd^{2+}

concentration (such as 0.1 mmol/L), the biodegradation enhancement effect of Cd^{2+} on crystalline atrazine was stronger than that of the DEHP-dissolved atrazine. As mentioned, the main reason could be the lower bioavailability of atrazine in DEHP. Correspondingly, the promotion of atrazine biodegradation in the presence of the Cd^{2+} concentration of 0.05–1.5 mmol/L could be attributed to the enhanced atrazine catabolism (Figure 6). Low concentrations of heavy metal ions could stimulate the catabolism of organic substances [40]. Cu^{2+} concentrations of 15 and 2 mg/L stimulated the degradation of decabromodiphenyl ether and benzo[a]pyrene, respectively [41,42]. An assessment of Cd pollution in arable soil in China showed that the average and maximum Cd concentrations were 0.0024 and 1.36 mmol/kg, respectively [6], both lower than the 1.5 mmol/L shown in the present study. Therefore, ST11 is not affected by soil Cd when it degrades atrazine in arable soils.

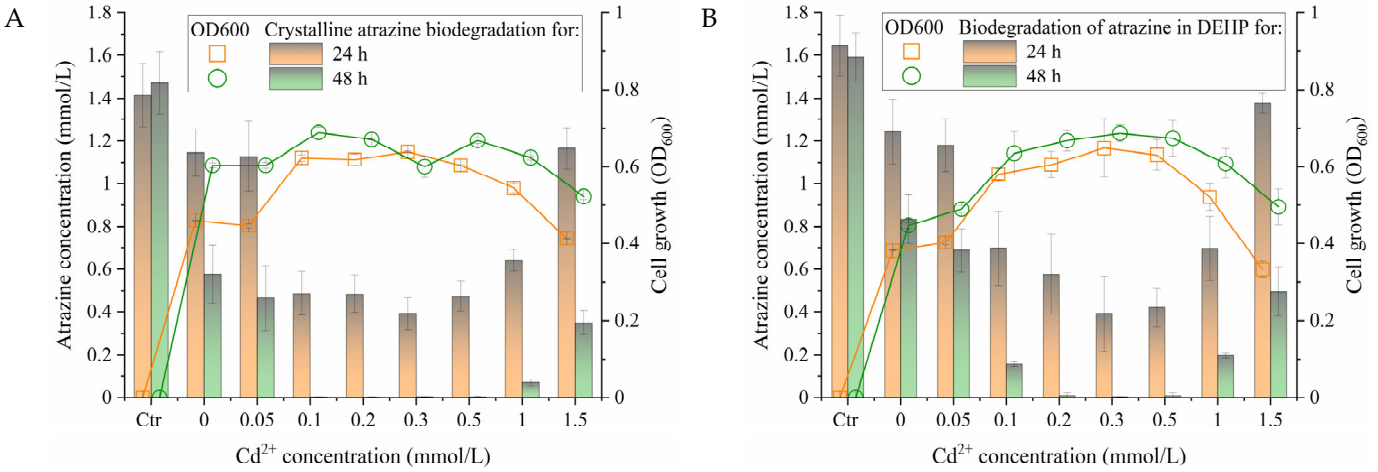

Figure 6. Effect of Cd^{2+} of different concentrations on bacterial growth and atrazine biodegradation. (**A**) crystalline atrazine; (**B**) DEHP dissolved atrazine. The initial concentration of atrazine was 1.85 mmol/L. Ctr: control samples with no cells inoculated. For each biological flask, 1 mL of bacterial suspension (OD_{600} 1.0) was inoculated. At an interval of 24 h, the growth was monitored spectrophotometrically by measuring OD_{600} and the residual atrazine was detected. Data points represent the mean of three replicates and error bars show the standard deviation.

As is well known, Cd, similar to other heavy metals, such as copper, zinc, and lead, has biological toxicity [5]. Cd could attach to proteins with sulfhydryl functional groups or glutathione, thus interfering with the synthesis of cysteine or directly damaging DNA [43]. In addition, Cd could easily penetrate the cell membrane of bacteria, causing the emission of substances in cells and affecting the metabolic process of cells [44]. The results of the present study showed that Cd inhibited and stimulated the growth of ST11 using soluble substrates (Cd^{2+} concentration of 0.5–1.5 mmol/L) and hydrophobic substrate atrazine (Cd^{2+} concentration of ≤1.5 mmol/L), respectively. ST11 cells have negligible Cd adsorption capacity, which may be one of the reasons why they could resist high Cd concentration. In addition, the antagonistic effect of hydrophobic organics on Cd could be used to mitigate Cd effect on soil living organisms [45]. The results of the present study are consistent with that of the previous study. The biodegradation of crystalline atrazine and NAPL-solubilized atrazine by ST11 could not be affected by Cd^{2+} with concentrations up to 1.5 mmol/L.

3. Materials and Methods

3.1. Chemicals

Atrazine (product number TCI-A1650, 97.0%), $CdCl_2 \cdot 2.5H_2O$ (≥99.0%), ethyl acetate (>99%), and methanol (>99%) were purchased from Sinopharm (Shanghai, China). DEHP (≥99.5%) was obtained from Aladdin (Shanghai, China). 2-(5-Bromo-2-pyridylazo)-5-(diethylamino) phenol (5-Br-PADAP, 97%) and Triton X-114 (laboratory grade) were

received from Sigma-Aldrich (Shanghai, China). The other reagents and solvents were of analytical grade and used directly. The stock solution of atrazine was prepared by dissolving atrazine in dichloromethane (92.73 mmol/L).

3.2. Microorganism and Culture Conditions

Soil samples were collected by hand-picking in a 5–10 cm soil layer with a modified sampling technique [46]. Each sample was placed in a portable icebox and then transferred to the laboratory. An efficient atrazine-degrading bacterium *Arthrobacter* sp. ST11 was isolated from herbicide-polluted soil. The bacterium was cultivated on LB medium (10 g tryptone, 5 g yeast extract, and 10 g NaCl per liter of Milli-Q deionized water; pH 6.8; stored at room temperature after sterilization) at 30 °C, with shaking at 150 rpm for 18 h. The cells were harvested by centrifugation at 6380× *g* for 10 min at 4 °C and washed twice with mineral salt medium (MSM, 5.8 g Na_2HPO_4, 0.9 g KH_2PO_4, 0.2 g $MgSO_4 \cdot 7H_2O$, and 1 mL of trace element solution per liter of Milli-Q deionized water; pH 6.5). The composition of the trace element solution was as follows: 0.4 g $Na_2B_4O_7 \cdot 10H_2O$, 0.5 g $Na_2MoO_4 \cdot 2H_2O$, 0.8 g $CuSO_4 \cdot 5H_2O$, 2 g $FeSO_4 \cdot 7H_2O$, 2 g $MnSO_4 \cdot H_2O$, 10 g $ZnSO_4 \cdot 7H_2O$, and 5 g EDTA disodium per liter of Milli-Q deionized water; pH 6.5. The cells were resuspended with MSM, and the optical density at 600 nm (OD_{600}) was adjusted to 1.0.

3.3. Cd^{2+} Resistance and Growth Curve of ST11

The resistance of ST11 to Cd^{2+} was investigated. The Cd^{2+} concentrations ranged from 0 to 1.5 mmol/L for ST11. The cells were cultured with an LB medium without Cd^{2+} at 30 °C at 150 rpm in darkness for 24 h. After the cells were washed three times and resuspended with Milli-Q deionized water, 1 mL of cell culture (OD_{600} = 1.0) was transferred into 150 mL sterilized flasks containing 30 mL of LB medium with various Cd^{2+} concentrations. The flasks were incubated at 150 rpm in darkness at 30 °C for 48 h. Cell growth was recorded by measuring the OD_{600} of the medium. A survival curve was generated using the growth changes in cultures supplemented with various concentrations of Cd^{2+} compared with no-Cd^{2+}-treated control samples. The regular interval for the biodegradation experiment was 48 h.

3.4. Cd^{2+} Adsorption Experiment

The adsorption of ST11 cells for Cd^{2+} was examined. After culturing in an LB medium for 60 h, ST11 cells were harvested through centrifugation (H2050R, Xiangyi, China) at 6380× *g* and 4 °C for 10 min. The cells were then washed three times with Milli-Q deionized water to remove the residual medium. The obtained wet cells were used directly for Cd^{2+} adsorption without further treatment. An aqueous solution of Cd^{2+} with a concentration of 0–12 μmol/L was prepared, and 10 mL of the solution was added to a 15 mL glass test tube. Furthermore, the wet cells were added to the tube, and the cell concentration in the aqueous solution was set as OD_{600} = 1.0. The above solutions were shaken at 25 °C and 150 rpm for 1 h. After centrifugation, the residual amount of Cd^{2+} in the supernatant was detected.

3.5. Biodegradation of Crystalline Atrazine

For experiments with crystalline atrazine, the bacterium was grown at 30 °C and 150 rpm in 150 mL-triangular flasks containing 30 mL of MSM supplied with different concentrations of atrazine as the sole source of carbon, nitrogen, and energy. A total of 1 mL of resuspended cells (OD600 1.0) was added into MSM. A certain volume of atrazine stock solution was evenly spread at the bottom of the pre-dried triangular flasks to avoid the influence of insoluble atrazine crystals on the absorbance detection of cells in the culture medium. A layer of homogeneous atrazine crystals was fixed at the bottom of the flask after the volatilization of dichloromethane for 12 h. In the process of cell culture, these crystals do not fall off from the bottom of the flask [47]. The regular interval for the biodegradation experiment was 48 h.

The biodegradation of atrazine was estimated indirectly as bacterial growth and atrazine content reduction. The bacteria were collected by centrifuging the culture medium at 6380× *g* and 4 °C for 10 min. The cells were washed three times with deionized water to remove the residual medium. The obtained wet cells were resuspended with MSM and the OD_{600} was determined. The complete biodegradation of atrazine was confirmed microscopically by the disappearance of atrazine crystals at the bottom of triangular flasks and by ethyl acetate extraction of the crystals after biodegradation and analysis of the extracts with an Agilent high-performance liquid chromatography (HPLC) system.

3.6. Biodegradation of Atrazine in NAPL

DEHP was used as the NAPL for determining the ST11 biodegradability of NAPL-dissolved atrazine. Experiments were performed in triangular flasks containing 24 mL of MSM and 6 mL of DEHP containing 9.25 mmol/L atrazine. The concentration of atrazine in the system was 1.85 mmol/L. For the inoculated sample, 1 mL of resuspended cells (OD600 1.0) was added to MSM. For the non-inoculated control experiment, no ST11 cells were added. The samples measured in triplicate were incubated at 30 °C on a rotary shaker at 150 rpm for 48 h.

The adherence of suspended bacteria to the liquid organic phase was tested. The method used was a modified assay of MATH [48]. DEHP (5 mL) and 20 mL of the cell suspension (OD_{600} 0.6) in MSM were vigorously homogenized in a test tube for 1 min by a vortex agitator (SA8, Thomas Scientific, Swedesboro, NJ. USA). After 2 h of equilibration, the cells' adhesion to DEHP was estimated from the loss of aqueous phase absorbance at 600 nm. Furthermore, optical imaging of the droplets at the oil-water interface was carried out to detect the location of bacterial cells by phase-contrast microscopy (Eclipse E200, Nikon, Tokyo, Japan).

3.7. Effect of Cd^{2+} Ions

The effect of Cd^{2+} ions on the metabolic activity of ST11 was investigated when atrazine was used as the substrate in crystal form or dissolved in DEHP. The concentrations of Cd^{2+} in MSM were set to 0–1.5 mmol/L. MSM separately supplemented with different concentrations of Cd^{2+} and 1.85 mmol/L atrazine was cultured at 30 °C, with shaking at 150 rpm for 48 h. Subsequently, the cell density of ST11 was monitored, and the residual amounts of atrazine were detected at 48 h. A sterile control culture without ST11 was included to assess the abiotic loss of atrazine. All measurements were carried out in triplicate.

3.8. Analytical Methods

The method for atrazine extraction was as follows: each triangular flask was added with 30 mL of ethyl acetate and shaken at 200 rpm for 1 h. Atrazine was extracted into the ethyl acetate layer. Furthermore, the aqueous and organic phases were separated using a separatory funnel. The organic phase was centrifuged at 6380× *g* for 10 min. A total of 500 µL of the supernatant was placed in a glass tube, and ethyl acetate was evaporated in an oven at 70 °C. The residue was extracted with 1 mL of methanol-water solution (85%, *v*/*v*). The extracts of the same sample were merged and filled into a 2 mL sample bottle after filtration through a 0.22 µm organic filter membrane, and then sealed and stored in a 4 °C refrigerator. Atrazine was detected using an HPLC system (Agilent 1260, Tokyo, Japan) with a UV detector. An Agilent HC-C18 (5 µm, 150 mm × 4.6 mm) column was used for the analysis of atrazine samples. Deionized water and methanol at a ratio of 85:15 were used as the mobile phase with a flow rate of 1 mL/min. The detection wavelength was 225 nm, and the retention time was 5.11 min. The detection limit of this method was 2 mg/L.

Cd^{2+} was determined by spectrophotometry after cloud point extraction [49]. In a 10 mL polystyrene tube, 0.2 mL of Cd^{2+} sample, 1 mL of NH_3-NH_4Cl buffer (pH 9.5), 0.53 mL of 5-Br-PADAP ethanol solution (0.2 g/L), and 0.6 mL of Triton X-114 solution (2%, *v*/*v*) were added successively. Furthermore, the liquid in the tube was diluted to 10 mL

with deionized water and mixed with a vortex agitator for 30 s. The mixed solution was heated at 45 °C for 15 min and then centrifuged at 1595× *g* for 5 min to accelerate the phase separation of the cloud point system. The solution after phase separation was quickly placed into an ice water mixture for quick freezing for 30 min to make the coacervate phase viscous. After the water phase was discarded, the coacervate phase was dissolved in 2 mL of absolute ethanol by shaking in a vortex agitator. Subsequently, these samples were detected at 560 nm via spectrophotometry (UV2700, Shimadzu, Tokyo, Japan). Each sample was measured at least three times.

4. Conclusions

In this study, an atrazine-degrading bacterial strain with Cd resistance from herbicide-polluted soil was isolated. Based on 16S rRNA gene sequence analysis and physiochemical tests, the bacterium was identified as *Arthrobacter* sp., and designated as strain ST11. The ST11 cells were grown in MSM culture with atrazine as the sole source of carbon, nitrogen, and energy. The strain could degrade atrazine in crystal form or present in DEHP as NAPL. To the authors' knowledge, this study was the first to report an *Arthrobacter* strain actively degrading crystalline and NAPL-dissolved atrazine with Cd resistance. Cd^{2+} with concentrations < 0.5 and 0.5–1.5 mmol/L did not affect or slightly inhibited the growth of ST11 in LB, respectively. Correspondingly, in the whole concentration range (0.05–1.5 mmol/L), Cd^{2+} promoted ST11 to degrade atrazine, whether in crystalline form or dissolved in DEHP. Refusal to adsorb Cd^{2+} may be the main mechanism of high Cd resistance in ST11 cells. Therefore, ST11 may be a potential candidate for the industrial elimination of atrazine from contaminated arable soils with Cd pollution. However, in-situ studies that examine the mechanisms of atrazine degradation and Cd resistance are necessary before this strain could be used in practice.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/catal12121653/s1, Table S1: Morphological, physiological, and biochemical properties of *Arthrobacter* sp. ST11; Figure S1: Photographs of colonies and cells of strain ST11: (A) photograph of ST11 colonies on LB solid media plate; (B) SEM photograph of ST11 cultured for 12 h; (C) SEM photograph of ST11 cultured for 36 h; Figure S2: The LC-UV chromatograms at 225 nm of atrazine standard (a), sample extract before atrazine degradation (b), and sample extract after atrazine degradation (c). The retention time of 5.081, 5.211, and 5.251 min is the absorption peak of atrazine. The retention time of 4.145 min was the absorption peak of a new metabolite.

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