Current Microbiology (2021) 78:1871–1881
https://doi.org/10.1007/s00284-021-02433-9

Isolation and Identification of *Pseudomonas* sp. Strain DY-1 from Agricultural Soil and Its Degradation Effect on Prometryne

Dong Liang[1] · Ming-yue Ding[1] · Chang-yixin Xiao[1] · Ya-wen Shen[1] · Yin-yue Wang[1] · Hai-tao Li[1] · Rong-mei Liu[1] · Ji-guo Gao[1]

Received: 26 August 2020 / Accepted: 16 February 2021 / Published online: 8 April 2021

Abstract

Prometryne is a widely used herbicide in China to control annual grasses and broadleaf weeds. However, the stability of prometryne makes it difficult to be degraded, which poses a threat to human health. This study presents a bacterial strain isolated from soil samples with a prometryne application history, designated strain DY-1. Strain DY-1, identified as *Pseudomonas* sp., is capable of utilizing prometryne as a sole carbon source for growth and degrading 100% of prometryne within 48 h from an initial concentration of 50 mg L^{-1}. To further optimize the degradation of prometryne, the prometryne concentration, temperature, pH, and salt concentration were examined. The optimal conditions for degradation of prometryne by strain DY-1 were an initial prometryne concentration of 50 mg L^{-1}, 30 °C, pH 7–8, and NaCl concentration of 200 mg L^{-1}. The same strain also degraded other s-triazine herbicides, including simetryne, ametryne, desmetryne, and metribuzin, under the same conditions. The biodegradation pathway of prometryne was established by isolating sulfoxide prometryne as the first metabolite and by the identification of sulfone prometryne and 2-hydroxy prometryne by liquid chromatography-mass spectrometry (LC–MS/MS). The results illustrated that strain DY-1 achieved the removal of prometryne by gradually oxidizing and hydrolyzing the methylthio groups. A bioremediation trial with contaminated soil and pot experiments showed that after treating the prometryne-contaminated soil with strain DY-1, the content of prometryne was significantly reduced ($P < 0.05$). This study provides an efficient bacterial strain and approach that could be potentially useful for detoxification and bioremediation of prometryne analogs.

Introduction

Prometryne [2-(methylthio)-4,6-bis(isopropylamino)-s-triazine], an herbicide belonging to the group of s-triazines, is a nonionic, weakly polar hydrophobic (log Kow > 2) compound [1], with average solubility in water. It is stable in slightly acidic, slightly alkaline, and neutral environments [2]. Prometryne has been extensively used as a pre- or post-emergence control of annual grasses and broadleaf weeds [3, 4]. The half-lives for the biodegradation of prometryne in aerobic and anaerobic soil have been reported as 1–3 months, respectively, and its production and use as an herbicide is expected to result in its release to the environment, triggering direct harm to humans through the food chain [5, 6]. In view of that, prometryne has been listed as a reproductive and developmental toxin by the U.S. Environmental Protection Agency (EPA) on their toxic substance release list, and is also identified as an endocrine disruptor on the EU priority list [7]. However, this chemical is still used in large quantities in the rice, sugarcane, and soybean growing regions of China because of its broad spectrum of weed control and high potency [8]. Therefore, the removal of prometryne from soil is an important aspect of ecological restoration. Previous research shows that prometryne can be removed by photodegradation, herbicide adjuvants, physical adsorbents, and molecularly imprinted polymers [7, 9–12]. Photodegradation can decrease the content of prometryne in soil [10, 11], but this process takes a long time. Using herbicide adjuvants may affect the biodegradation of recalcitrant compounds [7]. Physical adsorbents can remove prometryne from water [9], and molecularly imprinted polymers have been used to analyze herbicides at trace levels in media [12];

✉ Rong-mei Liu
liurongmei@neau.edu.cn

✉ Ji-guo Gao
gaojiguo1961@hotmail.com

[1] College of Life Science, Northeast Agricultural University, Harbin 150038, China

however, none of these approaches can solve the problem of prometryne in soil.

Microbial biodegradation is one of the key processes of herbicides attenuation in the environment [13]. This process has gradually become a focus of research and captured the attention of government officials due to its low cost, efficiency, lack of secondary pollution and other advantages [14]. Despite having been banned in several countries, prometryne is a widely used herbicide in China. Therefore, biodegradation may be an ideal strategy for the detoxification of prometryne from the environment. Many microorganisms that can degrade prometryne have been isolated and characterized, including *Leucobacter* sp. JW-1 [15], *Rhodococcus* sp. FJ1117YT [16], *Bacillus* sp. JUN7 [17], *Nicotinovorans* sp. HIM [18], *Nocardioides* sp. DN36 [19], and *Arthrobacter aurescens* TC1 [20]. To the best of our knowledge, only a few prometryne biodegradation pathways have been reported in the literature. Therefore, studies related to prometryne degradation are essential for a better understanding of prometryne-degrading bacteria.

In the present study, a new bacterial strain, *Pseudomonas* sp. DY-1, was isolated from prometryne-contaminated rice paddies and characterized by its efficient degradation of prometryne. The objectives of this study were to describe the degradation-related characteristics of this strain and its use in the bioremediation of prometryne-contaminated soil. Furthermore, the detailed pathway of prometryne metabolism was also elucidated.

Materials and Methods

Chemicals and Media

Prometryne ($C_{10}H_{19}N_5S$, 97%), simetryne ($C_8H_{15}N_5S$, 97%), desmetryne ($C_8H_{15}N_5S$, 98%), and metribuzin ($C_8H_{14}N_4SO$, 97%) were purchased from Harbin LIMIN Agrochemical Technology Ltd. Ametryne ($C_9H_{17}N_5S$, 97%) was purchased from Shanghai KALANG Technology Ltd. All of the other chemicals used in this study were analytical or HPLC-grade reagents that are available commercially.

Luria–Bertani (LB) medium contained 10.0 g L^{-1} tryptone, 5.0 gL^{-1} yeast extract, and 5.0 gL^{-1} NaCl, at pH 7.2. Minimal salt medium (MSM) contained 1 gL^{-1} K_2HPO_4, 1 gL^{-1} KH_2PO_4, 1 gL^{-1} NH_4NO_3, 0.2 gL^{-1} $MgSO_4{\cdot}7H_2O$, 0.2 gL^{-1} NaCl, and 1% trace element solution (TES), at pH 7.2. TES contained 0.4 gL^{-1} $Na_2B_4O_7{\cdot}10H_2O$, 0.5 gL^{-1} $Na_2MoO_4{\cdot}2H_2O$, 0.8 gL^{-1} $CuSO_4{\cdot}5H_2O$, 2 gL^{-1} $FeSO_4{\cdot}7H_2O$, 2 gL^{-1} $MnSO_4{\cdot}H_2O$, 10 gL^{-1} $ZnSO_4{\cdot}7H_2O$, and 5 gL^{-1} EDTA, at pH 7.2. Solid media plates received a concentration of 1.5% agar.

MSM with different concentrations of prometryne was used in enrichment, isolation, and degradation experiments, while LB medium was used to purify and preserve the bacterial strains.

Enrichment and Isolation of Prometryne-Degrading Bacteria

Soil samples were collected from the top 0–20 cm of a rice paddy that had been treated with prometryne for over 5 years in northwestern Heilongjiang Province, China. All of the samples were stored at 4 °C for no more than 2 weeks after being passed through a 2.0-mm sieve.

For the whole experiment, bacterial cells were inoculated at 5% (*v*/*v*) into a 250-mL flask containing 100 mL of MSM unless otherwise stated. Soil samples were added to MSM supplemented with 50 mg L^{-1} prometryne as the sole carbon source. Initially, the liquid culture was incubated in the dark on a rotary shaker at 30 °C and 160 rpm for 7 days. After that the culture was transferred to fresh MSM containing 100 mg L^{-1} to start the second soil enrichment. This process was repeated four times until the prometryne concentration reached 200 mg L^{-1}. Afterward, 100 µL of serial dilutions (10^{-4}, 10^{-5}, and 10^{-6}) of the enrichment culture were spread onto LB agar plates and incubated in the dark for 48 h at 30 °C. On the basis of morphological features, different bacterial colonies were picked, purified, and tested for their prometryne-degrading ability. All of the above were implemented to identify favorable conditions for prometryne-degrading bacteria and a growth-limiting culture for other soil microorganisms. The final isolated strain was named DY-1, which was preserved at − 80 °C in LB medium with 30% sterile glycerol for further assay.

Identification of the Isolated Strain

Strain DY-1 was identified on the basis of its morphological, physiological, and biochemical properties [21] (with reference to Bergey's Manual of Determinative Bacteriology) and its 16S rRNA gene sequence. The morphology of the strain was observed by electron microscopy, and the total genome was extracted using a Bacterial Genomic DNA Kit (Cat No. CW0552) according to the manufacturer's protocols. The extracted DNA was amplified using the universal primer set 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) of the bacterial 16S ribosomal RNA (rRNA) genes [22]. The PCR products were examined using 0.7% agarose gel electrophoresis and purified using an Axygen® AxyPrep DNA Gel Extraction Kit (Cat No. AP-GX-250). The purified products were sequenced by Jilin Comate Bioscience Co., Ltd. To align 16S rRNA gene sequences, a Basic Local Alignment Search Tool (BLAST) was used to identify sequences, Clustal X2 was used for alignment, and Molecular Evolutionary Genetics Analysis (MEGA) version 7.0 [23] was used to construct

the phylogenetic tree with the maximum livelihood method and the Kimura two-parameter distance model to calculate each distance. In each case, bootstrap values were calculated based on 1000 replications.

Growth and Degradation Conditions of the Prometryne-Degrading Strain

Strain DY-1 was incubated in LB medium at 30 °C for 24 h, and then the bacteria were harvested by centrifugation at 8000 rpm for 5 min at room temperature. The supernatant was discarded, using sterilized water to suspend the bacteria. The optical density at 600 nm (OD_{600}) of all the cultures was adjusted to 1.0 to determine the growth and biodegradation ability of strain DY-1. Following this, the samples that were already transferred into MSM were incubated in the dark at 30 °C and 160 rpm on a rotary shaker.

To investigate the effects of the concentrations of prometryne on the growth and prometryne biodegradation of strain DY-1, the concentrations were adjusted to 50, 100, 200, 300, 400, and 500 mg L^{-1} for different treatments, and the mixtures were incubated in the dark for 48 h at pH 7.0 and 30 °C. To determine the effects of temperature on strain DY-1, cultures containing 50 mg L^{-1} prometryne with a pH adjusted to 7.0 were incubated in the dark for 48 h at different temperatures of 15, 20, 25, 30, 35, 40, and 45 °C. The pH values of different cultures (containing 50 mg L^{-1} prometryne) were adjusted to 4–12 to analyze the effects of pH on strain DY-1 and incubated in the dark for 48 h at 30 °C. To study the effects of NaCl concentrations on strain DY-1, NaCl was added into different cultures (containing 50 mg L^{-1} prometryne) to final concentrations of 200, 400, 600, 800, and 1000 mg L^{-1}, and the mixtures were incubated in the dark for 48 h at pH 7.0 and 30 °C. The cultures without NaCl were used as negative controls. All of the treatments were repeated three times.

Prometryne extraction was done accordance with Liu et al. [15] with minor modifications. Samples from the cultures were collected in sterilized centrifuge tubes every 4 h. An equal volume of dichloromethane was added to the tubes and shaken to mix well, and the tubes were centrifuged at 10,000 rpm for 5 min and incubated for 20 min at room temperature. After the samples were delaminated, the supernatant was discarded, and the organic phase was transferred into the round-bottom flasks. Then, all of the flasks were evaporated in a rotary evaporator and dissolved in 1 mL of methanol.

The growth of DY-1 was analyzed using a spectrophotometer (U-2910; HITACHI, Japan) to measure the absorbance at 600 nm of the cultures, while HPLC (Waters 600; Waters, USA) was used to quantify the concentration of prometryne. An Agilent TC-C_{18} separation column (4.6 × 250 mm, 5 μm) (Cat No. 518925-902) was used at the stationary phase. The mobile phase was methanol: water (80:20, v/v), the flow rate was 1.0 mL min^{-1}, and the UV detection was set to 216 nm.

Identification of the Metabolites Products

To analyze the intermediate metabolites during prometryne degradation by strain DY-1, 10 mL of the cell suspension (OD_{600}, 1.0) was added to 200 mL of MSM containing 50 mg L^{-1} prometryne with the pH adjusted to seven and incubated in the dark for 48 h at 30 °C and 160 rpm on a rotary shaker. Then, prometryne was extracted using the method described in the previous section.

The metabolite products were determined by liquid chromatography tandem-mass spectrometry (LC–MS/MS, LCQ Deca XP; Thermo Finnigan, USA). A Waters BEH C_{18} column (3.9 mm × 100 mm × 1.7 μm) was used to separate the transformation products. The sample injection volume was 25 μL. The mass spectrometry mobile phase consisted of acetonitrile and water, and the flow rate was 0.1 mL min^{-1}. Gradient elution was performed under the following conditions successively: 0–2 min, 10% acetonitrile, and 90% water; 2–24 min, 70% acetonitrile, and 30% water; and 24–36 min, 10% acetonitrile, and 90% water. The mass spectrometry mode was SIR ion scanning with an ESI (+) source voltage of 3.5 kV, and capillary voltage of 24 V, capillary temperature of 25 °C.

Determination of the Ability of Strain DY-1 to Degrade Other 3-Triazine Herbicides

The cell suspension (OD_{600}, 1.0) was added to the four MSM containing 50 mg L^{-1} simetryne, ametryne, desmetryne, and metribuzin. All of the cultures were incubated in the dark for 48 h at 30 °C and 160 rpm on a rotary shaker, and then herbicides were extracted for analysis. The degradation dynamics were determined by HPLC, with a noninoculated suspension as a control.

Bioremediation of the Prometryne-Contaminated Soil

Soil samples were collected from the top 0–20 cm of the experimental farm at Northeast Agricultural University, which had not experienced contamination by prometryne or other *s*-triazine herbicides in the past 5 years. The samples were air-dried and passed through a 2.0-mm sieve. The prometryne (97%) was dissolved in methanol to obtain a 1 mg mL^{-1} solution. Then, to get 50 mg kg^{-1} of prometryne, the right amount of the solution was added to the dry soil and mixed. After the methanol was completely volatilized at room temperature, the treated soil was stored at 4 °C. Strain DY-1 was incubated in the two LB media until the number of living bacteria was 1×10^6 and 1×10^8 colony forming units

(CFU) mL^{-1}, and then the bacteria were harvested from the liquid culture by centrifugation at 8000 rpm for 5 min. The bacteria were suspended in sterile water to a density of approximately 1×10^6 and 1×10^8 CFU mL^{-1}, and then immediately sprayed onto the contaminated soil.

For reference, a blank control without prometryne or bacteria was used. Two of the experimental treatments contained 500 g of contaminated soil and 10 mL of strain DY-1 with different densities: (1) 1×10^6 CFU mL^{-1} and (2) 1×10^8 CFU mL^{-1}. Another treatment contained 500 g of the contaminated soil and 10 mL of sterile water. For each treatment, the soil moisture content was kept at 20%, and the temperature was kept at 25 °C. All the treatments were repeated three times.

Samples were collected from each group every 3 days, a double volume of methanol was added, and the mixture was stirred for 2 h, and then incubated for 12 h at room temperature. Next, the cultures were incubated for 1 h at room temperature on a rotary shaker and centrifuged at 8000 rpm for 10 min. Then the herbicide was extracted from the supernatant for determination of the concentration of prometryne by HPLC.

Pot Experiment

Methanol was used to dissolve the prometryne (97%). Soil samples from the previous section were added to the right amount of prometryne solution to obtain a concentration of 80 μg kg^{-1}. Strain DY-1 was incubated in LB medium until the number of living bacteria was 1×10^8 CFU mL^{-1}, and then the bacteria were harvested from the liquid culture by centrifugation at 8000 rpm for 5 min. The bacteria were suspended in sterile water to match the original volume and sprayed onto the contaminated soil immediately. Six kilograms of the treated soil was transferred into a pot ($23 \times 16.5 \times 20$ cm). Corn seeds were soaked at a constant temperature at 45 °C for 4 h; thereafter, their surfaces of them were disinfected. Subsequently, the germinated plants were wrapped in sterile gauze and sprayed with sterile water three times a day for 3 days at 28 °C. Each of the pots contained three germinated plants. Each group was cultured for 10 days at 28 °C and a 16 h/8 h light/dark cycle (1000 lx) in phytotron and sprayed with sterile water two times a day. After 10 days of cultivation, the morphological parameters of corn plants in every treatment group were measured, including the length and fresh weight of seedlings and roots.

To determine the influence of strain DY-1 and prometryne on the growth of corn, three treatments were applied: soil containing prometryne (experimental group, EG1), soil containing prometryne and strain DY-1 (EG2), and soil without prometryne or strain DY-1 (control group, CG). Each treatment group included six replicates to ensure accuracy.

Statistical Analysis

The data of pot experiment were carried out with six independent experiments, while of others were carried out with three. Each result shown in this paper is represented as the mean ± standard deviation (SD) or percentage of at least three replicated treatments. Statistical analysis was carried out with one-way analysis of variance (ANOVA) using SPSS software (version 25.0; IBM SPSS, Armonk, NY, USA) [24]. In addition, least significant differences (LSDs) were calculated or Games-Howell test was used for testing treatment effects at $P < 0.05$ [25].

Accession Number

The sequences of 16S rDNA of *Pseudomonas* DY-1 was submitted to the GenBank under the accession number KU054384.

Results and Discussion

Isolation and Identification of Prometryne-Degrading Strains

A strain (named DY-1) was isolated from soil after enrichment culturing. The cells of strain DY-1 were gram negative, and colonies on LB agar plates were convex, cream-to-white, nontransparent and had a smooth surface and regular edges. Strain DY-1 tested negative in the methyl red, starch hydrolysis, liquefaction of gelatin, and Voges-Proskauer tests and tested positive in the oxidase and nitrate reductase tests. On the basis of the 16S rRNA gene sequence of strain DY-1, a phylogenetic tree was constructed, as shown in Fig. 1, which indicated that strain DY-1 showed 98.66% similarity with *Pseudomonas resinovorans* ATCC14235^T (NR 112062). The isolated strain, DY-1, was identified as a species of *Pseudomonas* in accordance with all the above.

Optimization of the Growth and Degradation Conditions of Strain DY-1

The effects of different substrate concentrations on the growth and degradation ability of strain DY-1 are shown in Fig. 2a. In previous studies, Liu et al. [15] chose 50, 200, and 400 mg L^{-1} as initial prometryne concentrations to determine the degradation rate of prometryne, while Zhang et al. [26] used 10, 20, 50, 80, and 100 mg L^{-1} to analyze the degradation characteristics of atrazine. To study the prometryne degradation characteristics of strain DY-1 more clearly, the initial concentration range of prometryne was further expanded on the basis of previous studies. At initial prometryne concentrations of 50 and

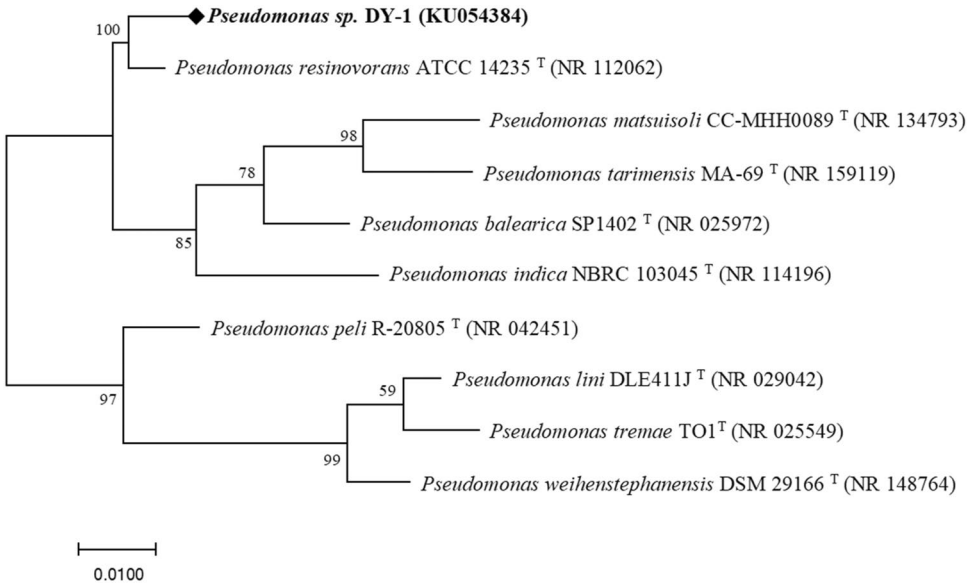


Fig. 1 Phylogenetic analysis of strain DY-1 constructed based on the 16S rDNA sequences with maximum livelihood method. The bootstrap values were calculated based on 1000 replications

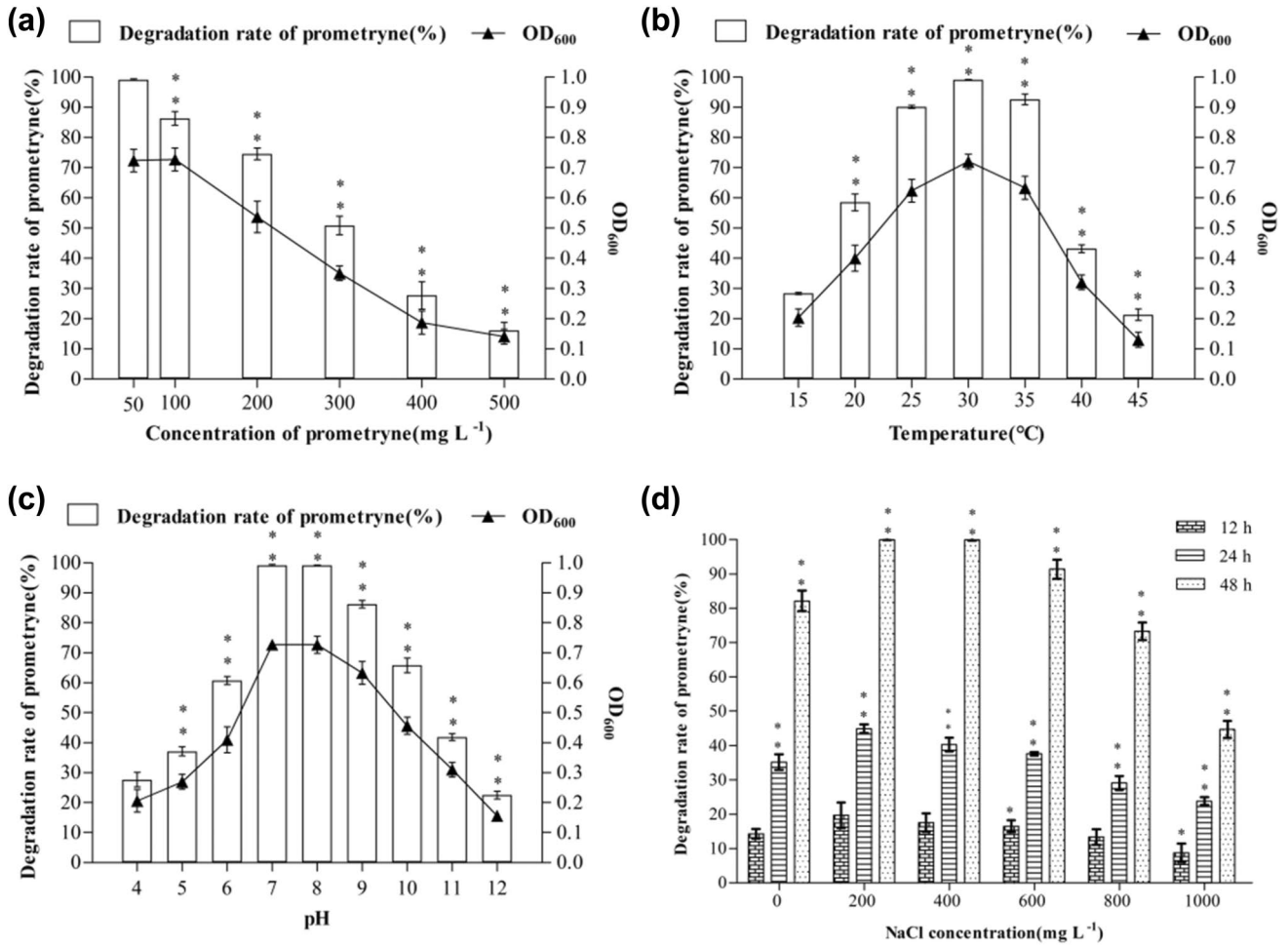


Fig. 2 The effects of environmental factors on prometryne degradation by stain DY-1 after 48 h incubation ($n=3$). **a** Prometryne concentration, **b** temperature, **c** pH, and **d** NaCl concentration. Optical density at 600 nm indicate OD_{600}. Asterisk and double asterisk denote significant effect of environmental factors at $P<0.05$ and $P<0.01$, respectively

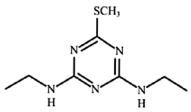
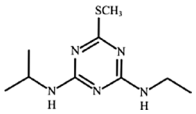
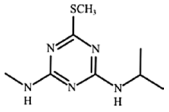
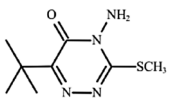
100 mg L^{-1}, the removal efficiencies were 100 and 85% after 48 h, respectively. However, it is worth mentioning that the growth rate was rose considerably at 100 mg L^{-1}, being slightly higher than that under the former. This result demonstrated that the low concentration of prometryne may not be capable of providing enough both energy and carbon to allow the bacteria to grow normally. Furthermore, strain DY-1 could efficiently degrade over 50% of prometryne within 48 h at a concentration of 300 mg L^{-1}. When the initial prometryne concentrations reached 400 and 500 mg L^{-1}, both the growth and degradation ability of strain DY-1 were less pronounced, which indicated that high concentrations of prometryne inhibited the growth of strain DY-1, giving rise to a decrease in the degradation ability of strain DY-1. Although the initial prometryne concentration was high, the strain did show a positive effect on degradation. Similar trends were observable in studies of *Bacillus* sp. reported previously: Wang et al. [27] suggested that the biodegradation ability of HB-6 declines from 90 to 20% when the atrazine concentration is 500 mg L^{-1} and that only 100 mg L^{-1} is beneficial for biodegradation; Wang et al. [28] showed that a lower buprofezin concentration can promote the degradation of BF-5; and Lu et al. [29] concluded that the nicosulfuron capability degradation of YB1 is significantly higher at 0.5–2.0 mg L^{-1} than at 5–10 mg L^{-1}. In China, a strict maximum residue limit of prometryne is set at 0.02 mg kg^{-1} for various agricultural products [30]. Strain DY-1 could degrade prometryne at a concentration of 500 mg L^{-1}, which is much higher than at 0.02 mg kg^{-1}. Moreover, only a few strains could degrade herbicides at a concentration of 500 mg L^{-1}, indicating that strain DY-1 had good tolerance to prometryne and excellent application prospects.

The efficiency of microbial bioremediation depends on the temperature and pH of the contaminated areas [31]. As shown in Fig. 2b, the optimal temperature for prometryne degradation of strain DY-1 was 30 °C, a with degradation efficiency reaching 100%. Moreover, the degradation rates of prometryne were very similar at 25 and 35 °C with over 90% degradation within 48 h of cultivation. At 15, 20, 40, and 45 °C, the rates decreased compared with those at the above temperatures. Furthermore, strain DY-1 could hardly grow when the temperature was below 20 °C or above 40 °C, suggesting that temperature affected the cell concentration of bacteria, which in turn affected the degradation rate of prometryne. Previous studies of s-triazine herbicide biodegradation have drawn similar conclusions. For instance, Wang et al. [32] found that the atrazine degradation rate of *Arthrobacter* sp. DAT-1 increases with temperature in a range from 10 to 35 °C, and the results of Zhao et al. [33] and Zhang et al. [26] indicate that the rates of *Pseudomonas* sp. ZXY-1 and *Klebsiella variicola* FH-1 reached their maximum levels at 25–35 and 25–30 °C, respectively.

The degradation of prometryne by strain DY-1 under a series of pH values is illustrated in Fig. 2c. The degradation efficiency reached 100% at pH 7.0 and 8.0 and was 87, 68, 60, 43, and 37% at pH 9.0, 10.0, 6.0, 11.0, and 15.0, respectively. The results indicated that the prometryne degradation efficiency of strain DY-1 was higher under neutral or weakly alkaline conditions than under strongly acidic or alkaline conditions. Liu et al. [15] reported that a neutral pH was the most favorable for prometryne degradation by *Leucobacter* sp. JW-1, which was similar to our results.

Figure 2d shows the effects of different NaCl concentrations on the prometryne degradation capability of strain DY-1. In the control group without NaCl, the prometryne removal percentage was 82.1% after 48 h. Concentrations of 200, 400, and 600 mg L^{-1} were more effective for removing prometryne than the control group. When the concentrations were 200 and 400 mg L^{-1}, both of the removal percentages were 100%, while at 600 mg L^{-1} it was 91.4%. However, the prometryne removal percentages were 73.3 and 44.7% under NaCl concentrations of 800 and 1000 mg L^{-1}, respectively, rates lower than those of the control. The results showed that an appropriate concentration of salt in the culture was helpful to improve the degradation efficiency of prometryne by strain DY-1. Similar to our results, Shir et al. [34] reported that the atrazine biodegradation rate of *Ochrobactrum oryzae* was decreased without NaCl, and Zhao et al. [35] indicated that atrazine degradation by *Arthrobacter* sp. ZXY-2 was improved with a proper salt concentration.

Table 1 Degradation of s-triazine compounds by strain DY-1

Herbicide	Structure	Degradation percentage (%)
Simetryne		90.2
Ametryne		87.8
Desmetryne		93.6
Metribuzin		38.2

Degradation Spectrum of Strain DY-1

Table 1 indicates that strain DY-1 could grow on the screening media with simetryne, ametryne, desmetryne, and metribuzin at a concentration of 50 mg L^{-1}, proving that strain DY-1 could degrade the above s-triazine herbicides containing methylthio (–SCH_3). This substrate specificity may be related to the structures of the methylthio group, and the same conclusion was reached in previously for *Rhodococcus* sp. FJ1117YT [16], *Bacillus cereus* JUN7 [17], *Leucobacter* sp. JW-1 [15], and *Nocardioides* sp. C190 [36].

Biodegradation of Prometryne by Strain DY-1

The products of prometryne degradation by strain DY-1 were identified by LC–MS/MS, and the liquid quality of the

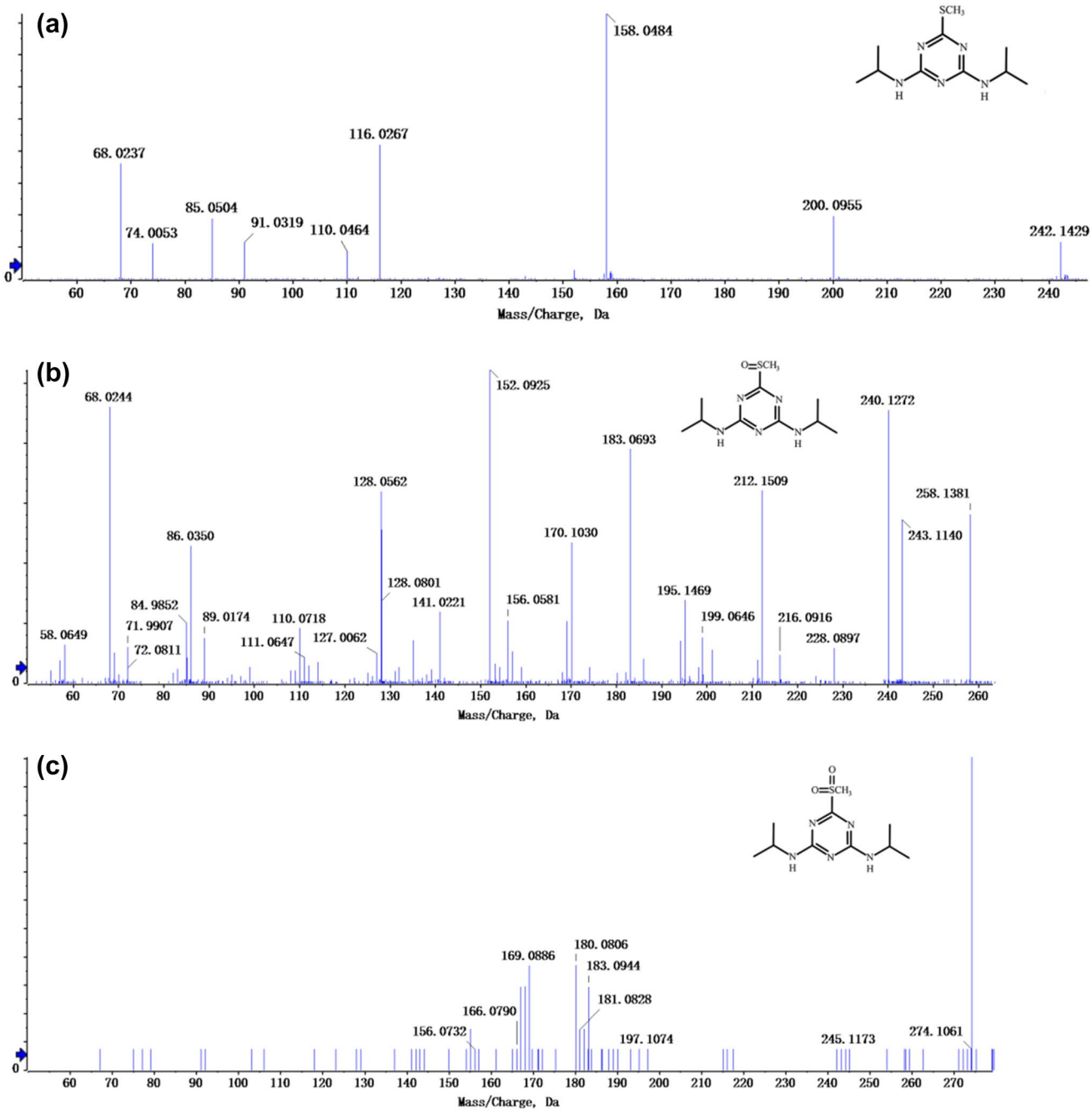


Fig. 3 LC–MS/MS identification of the products of prometryne degradation by strain DY-1. **a** Prometryne, **b** sulfoxide prometryne, **c** sulfone prometryne, **d** 2-hydroxy prometryne, and **e** mass spectra of prometryne from NIST database [37]

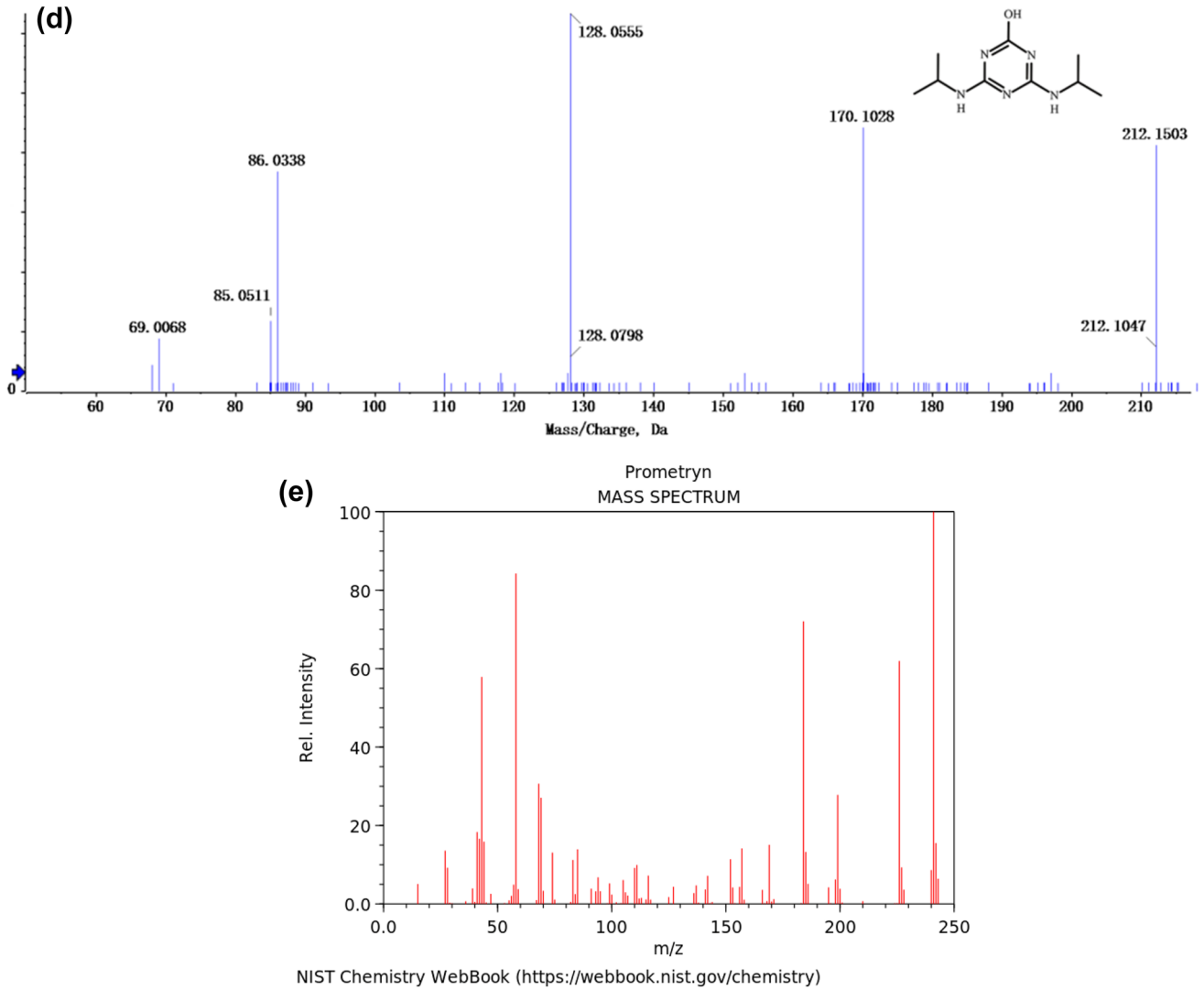


Fig. 3 (continued)

culture medium samples at different times was determined by total ion chromatogram (TIC). The mass-to-charge ratios (*m*/*z*) of the metabolites are shown in Fig. 3. The structure of prometryne was confirmed at *m*/*z* 242.1429 (Fig. 3a) on the basis of its protonated molecular ion $[M+H]^+$ and the National Institute of Standards and Technology (NIST) library identification program (Fig. 3e) [37]. Based on the mass spectra, the major fragments of metabolite I exhibited at *m*/*z* 216.0916, 183.0693, and 170.1030 (Fig. 3b), and the formula of metabolite I was presumed to be $C_{10}H_{19}N_5SO$. In addition, compared with prometryne, the peaks of the $[M+H]^+$ of metabolite II (Fig. 3c) at *m*/*z* 274.1061 implied that an additional oxygen atom emerged on the parent molecule. The same phenomenon could be observed between metabolites I and II, and then the latter was hydrolyzed rapidly, resulting in metabolite III, the formula of which might be $C_9H_{17}N_5O$ (Fig. 3d).

In general, the molecular formulas of the three metabolites could be $C_{10}H_{19}N_5SO$ with a retention time (*RT*) of 8.0655 min, $C_{10}H_{19}N_5SO_2$ (*RT* = 12.9335 min), and $C_9H_{17}N_5O$ (*RT* = 8.4012 min). Combined with the chemical structure of prometryne, the results indicated that the three substances were sulfoxide prometryne, sulfone prometryne, and 2-hydroxy prometryne. Nevertheless, the accumulation of 2-hydroxy prometryne was observed, suggesting that strain DY-I might not be capable of metabolizing 2-hydroxy prometryne. It is possible claimed that at the beginning, strain DY-1 modifies prometryne by oxidation, and then the methylthio group is substituted by the hydroxy group, according to the molecular ion (M^+) peaks of metabolites I (Fig. 3b) and metabolite III (Fig. 3c).

Figure 4 shows a schematic diagram of the degradation pathway of strain DY-1 based on these metabolites.

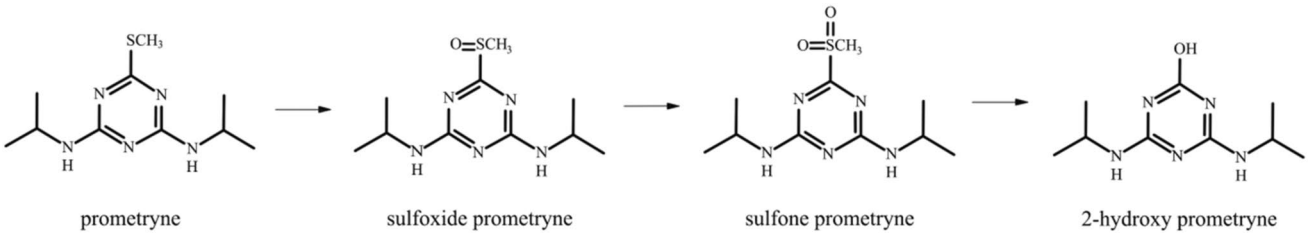


Fig. 4 Proposed degradation pathway of prometryne degradation by strain DY-1 based on the presumed metabolites

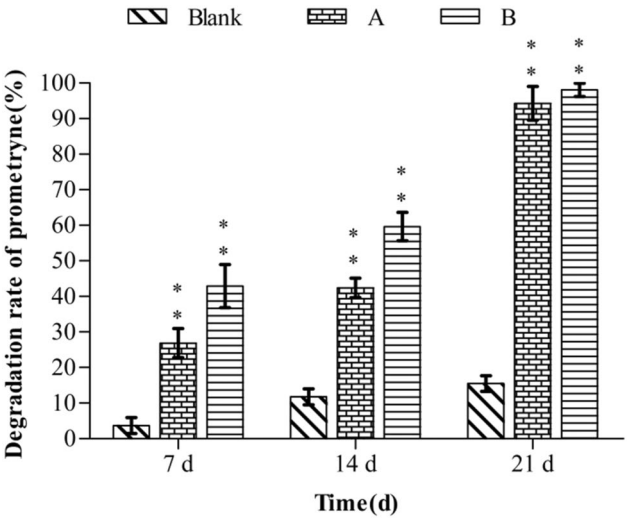


Fig. 5 The degradation rate of prometryne in the soil by different colony numbers of strain DY-1 after incubation for 7, 14, and 21 days at 25 °C ($n=3$). *Blank* noun, *A* 1×10^6 CFU mL^{-1}, and *B* 1×10^8 CFU mL^{-1}. Colony forming units indicate CFU. Asterisk and double asterisk denote significant effect of different colony numbers (compared to blank control) at $P<0.05$ and $P<0.01$, respectively

Similar to strain DY-1, strains FJ1117YT [16] and JUN7 [17] degrade methylthio s-triazine by oxidation and hydrolysis.

Soil-Bioremediation and Detoxification by Strain DY-1

The degradation dynamics of prometryne at 50 mg kg^{-1} in soil are shown in Fig. 5. It could be seen that 94.3 and 98.1% of the prometryne was removed by treatment with 1×10^6 and 1×10^8 CFU mL^{-1} of strain DY-1, respectively, after incubation at 25 °C for 21 days, and for the blank control, chemical hydrolysis might be the reason why approximately 15.5% of the prometryne was dissipated. There was little difference in degradation between the two bacterial concentrations after 21 days, which indicated that the bacterial concentration was not important, but that bacteria were present. Therefore, this might be due to the synergistic effect between the low bacterial concentration and the microorganisms contained in the soil, which accelerated the degradation process of prometryne in the soil. Previous studies have resulted in similar conclusions, for instance, *Arthrobacter* sp. DNS10 can degrade 67.7% of atrazine (20 mg kg^{-1}) after 20 days [38], and *Arthrobacter* sp. LY-1, 98.3% of atrazine (100 mg kg^{-1}) after 14 days [39]. In China, overuse of herbicides in agriculture led to the accumulation of *s*-triazine at approximately 2.89–3.81 mg kg^{-1} [40], prompting the search for a method to address this phenomenon. This is the first step to remedy this trend. The concentration of prometryne in the field is much lower than that used in this study (50 mg L^{-1}). Therefore, it is practically feasible to remove prometryne residues in nature using strain DY-1.

The pot experiment used corn as an indicator to evaluate the detoxification ability of strain DY-1 for

Table 2 The growth of corn in the soil treated with prometryne, the mixture of prometryne and strain DY-1, and the control soil ($n=6$)

	Length of seeding (cm)	Length of root (cm)	Fresh weight of seeding (g)	Fresh weight of root (g)
CG	19.73 ± 0.036	14.28 ± 0.048	5.42 ± 0.068	2.15 ± 0.054
EG1 (prometryne)	7.23 ± 0.057**	4.83 ± 0.010**	1.30 ± 0.056**	0.97 ± 0.052**
EG2 (prometryne + DY-1)	19.09 ± 0.074**	13.51 ± 0.089**	4.36 ± 0.057**	2.08 ± 0.041*

Data obtained after 14 days of treatment are given as mean ± standard deviation (SD)

CG control group, *EG* experimental group

*, **Denote significant effect of different treatments (compared to CG) at $P<0.05$ and $P<0.01$, respectively

prometryne-contaminated soil. After cultivation for 14 days, corn growth data were obtained under different treatments, and the data are shown in Table 2. The data showed significant improvements in the seedling length, root length, seedling fresh weight and root fresh weight of corn of the EGs compared with the CG ($P < 0.05$). Moreover, compared with the CG, the measured morphological parameters of the EG1 decreased by 36.6, 33.8, 23.9, and 45.1%, respectively ($P < 0.01$). This signified that prometryne had toxic effect on corn growth, and it might become a pollutant of nontarget crops after decomposition. The measured morphological parameters in EG2 were higher than EG1, and were 96.8, 94.6, 80.4, and 96.7% of the control values ($P < 0.05$). The results highlighted that strain DY-1 had the ability to ameliorate the toxicity of prometryne to corn under laboratory conditions.

Conclusions

In summary, *Pseudomonas* sp. strain DY-1, which offers superior remediation capability for soil contaminated by prometryne at 30 °C and pH 7.0, was obtained using enrichment culture. The prometryne degradation by DY-1 occurred at a wide range of prometryne concentrations, and appropriate amounts of NaCl addition could help improve the prometryne removal efficiency. Additionally, the LC–MS/MS data suggested that during degradation by strain DY-1, prometryne was oxidized and hydrolyzed in MSM, yielding sulfoxide prometryne, sulfone prometryne, and 2-hydroxy prometryne successively. A bioremediation trial with contaminated soil and pot experiments indicated that DY-1 could degrade over 94% of the prometryne contained in soil (50 mg kg^{-1}) after incubation for 21 days at 30 °C. Moreover, the effects of strain DY-1 on crops during the degradation of prometryne were studied by a 21-day pot experiment, showing that strain DY-1 is capable of degrading prometryne into a form nontoxic to crops. The results of soil bioremediation and detoxification did not show a negative effect on soil and crops, which suggests that strain DY-1 is a promising candidate for the bioremediation of prometryne-contaminated environments, providing a new option for soil bioremediation and prometryne removal from crops. The experimental research results will hopefully prove useful for improvement of bioremediation, and provide a good starting point for discussion and further research.

Acknowledgments Special acknowledgment is given to all the participants in this research and their relatives for their cordial guidance and material support during the laboratory work. The authors thank the National Key R & D Projects (2017YFD0201200) and Heilongjiang Provincial National Science Foundation (C2016025) for support, and Springer Nature Research Editing Service for grammar editing.

Author Contributions The manuscript was written through contributions of all authors. All the authors have given approval to the final version of the manuscript. DL, HL, and RL contributed to conceptualization; DL and MD were involved in methodology; YS and YW performed experiment; DL was involved in writing—original draft preparation; DL and CX were involved in writing—editing; JG contributed to funding acquisition; HL and RL supervised the study.

Funding This research was funded by the National Key R & D Projects (Grant No. 2017YFD0201200) and Heilongjiang Provincial National Science Foundation (Grant No. C2016025).

Data Availability The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of Interest The authors declare that they have no conflicts of interest.

Informed Consent Written informed consent for publication was obtained from all participants.

References

1. Plakas KV, Karabelas AJ (2009) Triazine retention by nanofiltration in the presence of organic matter: the role of humic substance characteristics. J Membr Sci 336(1–2):86–100
2. United States Environmental Protection Agency Office of Prevention (1996) Pesticides and toxic substances, reregistration eligibility decision (RED)-prometryn. (7508W) EPA 738-R-95-033
3. Molin WT, Khan RA (1996) Differential tolerance of cotton (*Gossypium* sp.) cultivars to the herbicide prometryn. Pestic Biochem Physiol 56(1):1–11
4. Garba ZN, Zhou W, Lawan I, Zhang M, Yuan Z (2019) Enhanced removal of prometryn using copper modified microcrystalline cellulose (Cu-MCC): optimization, isotherm, kinetics and regeneration studies. Cellulose 26:6241–6258
5. Jiang L, Yang H (2009) Prometryne-induced oxidative stress and impact on antioxidant enzymes in wheat. Ecotoxicol Environ Saf 72(6):1687–1693
6. Cháfer-Pericás C, Herráez-Hernández R, Campíns-Falcó P (2006) On-fibre solid-phase microextraction coupled to conventional liquid chromatography versus in-tube solid-phase microextraction coupled to capillary liquid chromatography for the screening analysis of triazines in water samples. J Chromatogr A 1125(2):159–171
7. Pérez-Bárcena JF, Ahuatzi-Chacón D, Castillo-Martínez KL, Ruiz-Ordaz N, Galíndez-Mayer J, Juárez-Ramírez C, Ramos-Monroy O (2013) Effect of herbicide adjuvants on the biodegradation rate of the methylthiotriazine herbicide prometryn. Biodegradation 25(3):405–415
8. Zhou J, Hu F, Jiao J, Liu M, Li H (2012) Effects of bacterial-feeding nematodes and prometryne-degrading bacteria on the dissipation of prometryne in contaminated soil. J Soils Sediments 12(4):576–585
9. Grabka D, Raczyńska-Żak M, Czech K, Słomkiewicz PM, Jóźwiak MA (2015) Modified halloysite as an adsorbent for prometryn from aqueous solutions. Appl Clay Sci 114:321–329
10. Jiang C, Li XJ, Wang YR, Ma LY, Wang YK, Lu YC, Yang H (2017) Assessment of photodegradation of herbicide prometryn

in soil. Water Air Soil Pollut 228(4):135. https://doi.org/10.1007/s11270-017-3307-7
11. Boulahia K, Carol P, Planchais S, Abrous-Belbachir O (2016) *Phaseolus vulgaris* L. Seedlings exposed to prometryn herbicide contaminated soil trigger an oxidative stress response. J Agric Food Chem 64(16):3150–3160
12. Guo LJ, Qu JR, Miao SS, Geng HR, Yang H (2013) Development of a molecularly imprinted polymer for prometryne clean-up in the environment. J Sep Sci 36(24):3911–3917
13. Siripattanakul S, Wirojanagud W, McEvoy J, Limpiyakorn T, Khan E (2009) Atrazine degradation by stable mixed cultures enriched from agricultural soil and their characterization. J Appl Microbiol 106(3):986–992
14. Ye JY, Zhang JB, Gao JG, Li HT, Liang D, Liu RM (2016) Isolation and characterization of atrazine-degrading strain *Shewanella* sp. YJY4 from cornfield soil. Lett Appl Microbiol 63(1):45–52
15. Liu J, Hua R, Lv P, Tang J, Wang Y, Cao H, Wu X, Li Q (2017) Novel hydrolytic demethylthiolation of the s-triazine herbicide prometryn by *Leucobacter* sp. JW-1. Sci Total Environ 579:115–123
16. Fujii K, Takagi K, Hiradate S, Iwasaki A, Harada N (2007) Biodegradation of methylthio-s-triazines by *Rhodococcus* sp. strain FJ1117YT, and production of the corresponding methylsulfinyl, methylsulfonyl and hydroxy analogues. Pest Manag Sci 63(3):254–260
17. Harada N, Takagi K, Fujii K, Iwasaki A (2006) Transformation of methylthio-s-triazines via sulfur oxidation by strain JUN7, a *Bacillus cereus* species. Soil Biol Biochem 38(9):2952–2957
18. Aislabie J, Bej AK, Ryburn J, Lloyd N, Wilkins A (2005) Characterization of *Arthrobacter nicotinovorans* HIM, an atrazine-degrading bacterium, from agricultural soil New Zealand. FEMS Microbiol Ecol 52(2):279–286
19. Satsuma K (2010) Mineralization of s-triazine herbicides by a newly isolated *Nocardioides* species strain DN36. Appl Microbiol Biotechnol 86(5):1585–1592
20. Shapir N, Rosendahl C, Johnson G, Andreina M, Sadowsky MJ, Wackett LP (2005) Substrate specificity and colorimetric assay for recombinant *trzN* derived from *Arthrobacter aurescens* TC1. Appl Environ Microbiol 71(5):2214–2220
21. Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST (1994) Bergey's manual of determinative bacteriology. Williams and Wilkins, Baltimore
22. Frank JA, Reich CI, Sharma S, Weisbaum JS, Wilson BA, Olsen GJ (2008) Critical evaluation of two primers commonly used for amplification of bacterial 16S rRNA genes. Appl Environ Microbiol 74(8):2461–2470
23. Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 30:1870–1874
24. IBM Corp. Released (2017) IBM SPSS statistics for windows, version 25.0. IBM Corp, Armonk, NY
25. Steel R, Torrie JH (1997) Principles and procedures of statistics a biometrical approach. McGraw-Hill, New York
26. Zhang J, Liang S, Wang X, Lu Z, Sun P, Zhang H, Sun F (2019) Biodegradation of atrazine by the novel *Klebsiella variicola* strain FH-1. Biomed Res Int 2019:1–12
27. Wang J, Zhu L, Wang Q, Wang J, Xie H (2014) Isolation and characterization of atrazine mineralizing *Bacillus subtilis* strain HB-6. PLoS ONE 9(9):e107270
28. Wang G, Xu D, Xiong M, Zhang H, Li F, Liu Y (2016) Novel degradation pathway and kinetic analysis for buprofezin removal by newly isolated *Bacillus* sp. J Environ Manage 180:59–67
29. Lu XH, Kang ZH, Tao B, Wang YN, Dong JG, Zhang JL (2012) Degradation of nicosulfuron by *Bacillus subtilis* YB1 and *Aspergillus niger* YF1. Appl Biochem Microbiol 48(5):460–466
30. Liu C, Wang Y, Zhang L, Qin J, Dou X, Fu Y, Li Q, Zhao X, Yang M (2020) An integrated strategy for rapid on-site screening and determination of prometryn residues in herbs. Anal Bioanal Chem 412(3):621–633
31. Siddique T, Okeke BC, Arshad M, Frankenberger WT (2002) Temperature and pH effects on biodegradation of hexachlorocyclohexane isomers in water and a soil slurry. J Agric Food Chem 50(18):5070–5076
32. Wang Q, Xie S (2012) Isolation and characterization of a high-efficiency soil atrazine-degrading *Arthrobacter* sp. strain. Int Biodeterior Biodegrad 71:61–66
33. Zhao X, Wang L, Ma F, Bai S, Yang J, Qi S (2017) *Pseudomonas* sp. ZXY-1, a newly isolated and highly efficient atrazine-degrading bacterium, and optimization of biodegradation using response surface methodology. J Environ Sci 54:152–159
34. Shir MA, Dehghani M, Samaei MR (2016) Isolation of atrazine degrading bacteria in semi-salinity medium. J Health Sci Surveil Syst 4:121–129
35. Zhao X, Ma F, Feng C, Bai S, Yang J, Wang L (2017) Complete genome sequence of *Arthrobacter* sp. ZXY-2 associated with effective atrazine degradation and salt adaptation. J Biotechnol 248:43–47
36. Topp E, Mulbry WM, Zhu H, Nour SM, Cuppels D (2000) Characterization of s-triazine herbicide metabolism by a *Nocardioides* sp. isolated from agricultural soils. Appl Environ Microbiol 66(8):3134–3141
37. Linstrom PJ, Mallard WG (eds) (2018) NIST chemistry WebBook, NIST standard reference database number 69. National Institute of Standards and Technology, Gaithersburg, p 20899
38. Zhang Y, Zhang QY, Nan C, Ge SJ, Jiang Z, Hu M, Cao B (2015) Biodegradation of atrazine by free and immobilized cells of *Arthrobacter* sp. strain DNS10. Environ Eng Manag J 14(4):819–826
39. Li Y, Liang D, Sha J, Zhang JB, Li HT, Liu RM (2019) Isolating and identifying the atrazine-degrading strain *Arthrobacter* sp. LY-1 and applying it for the bioremediation of atrazine-contaminated soil. Polish J Environ Stud 28(3):1267–1273
40. Gao J, Song P, Wang G, Wang J, Zhu L, Wang J (2018) Responses of atrazine degradation and native bacterial community in soil to *Arthrobacter* sp. strain HB-5. Ecotoxicol Environ Saf 159:317–323