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Biodegradation of Buprofezin by *Rhodococcus* sp. Strain YL-1 Isolated from Rice Field Soil

Chao Li, Ji Zhang, Zhi-Guo Wu, Li Cao, Xin Yan,* and Shun-Peng Li

Key Lab of Microbiological Engineering of Agricultural Environment, Ministry of Agriculture, College of Life Science, Nanjing Agricultural University, Nanjing, Jiangsu Province, 210095, People's Republic of China

Supporting Information

ABSTRACT: A buprofezin-degrading bacterium, YL-1, was isolated from rice field soil. YL-1 was identified as *Rhodococcus* sp. on the basis of the comparative analysis of 16S rDNA sequences. The strain could use buprofezin as the sole source of carbon and nitrogen for growth and was able to degrade 92.4% of 50 mg L^{-1} buprofezin within 48 h in liquid culture. During the degradation of buprofezin, four possible metabolites, 2-*tert*-butylimino-3-isopropyl-1,3,5-thiadiazinan-4-one, *N*-*tert*-butyl-thioformimidic acid formylaminomethyl ester, 2-isothiocyanato-2-methyl-propane, and 2-isothiocyanato-propane, were identified using gas chromatography−mass spectrometry (GC−MS) analysis. The catechol 2,3-dioxygenase activity was strongly induced during the degradation of buprofezin. A novel microbial biodegradation pathway for buprofezin was proposed on the basis of these metabolites. The inoculation of soils treated with buprofezin with strain YL-1 resulted in a higher degradation rate than that observed in noninoculated soils, indicating that strain YL-1 has the potential to be used in the bioremediation of buprofezin-contaminated environments.

KEYWORDS: *Rhodococcus sp. YL-1, biodegradation, buprofezin, degradation pathway*

INTRODUCTION

Buprofezin (2-*tert*-butylimino-3-isopropyl-5-phenyl-1,3,5-thiadiazinan-4-one) is a broad-spectrum insect growth regulator that interrupts the development of immature insects by inhibiting chitin biosynthesis and subsequent cuticle deposition.[1−3] This compound has been widely used on tea, rice, potatoes, citrus fruit, cotton, and vegetables to control various pests, such as whiteflies, planthoppers, leafhoppers, and scales.[4−6] Because of its high hydrophobicity and stability in acid and alkali environments, buprofezin is easily adsorbed by soil particles, which may contribute to its long-term retention in soil.[7] Under aerobic field conditions, the half-life of buprofezin is in the range of 50−70 days, and under flooded field conditions, the half-life is approximately 36−104 days. Because of the widespread use of buprofezin in many areas, the residues present in the environment are a potential problem. The amount of buprofezin residue on lemons, oranges, and mandarins ranged from 0.05 to 0.69 mg kg^{-1} in New Zealand, Portugal, Australia, Italy, and Spain.[8] Buprofezin residues were also detected in plants and water.[9,10] In 2006, when buprofezin was recommended for use on exportable grapes, its residues were frequently detected.[11]

Buprofezin can be easily absorbed by the human body through the oral, dermal, and respiratory routes.[12] Although buprofezin is of low acute toxicity to mammals (acute rat oral LD_{50} was 1635 mg kg^{-1} in males), the removal of this pesticide from the environment has received considerable attention. Microbes play an important role in removing toxic substances from the environment, and microbial bioremediation is considered to be a cost-effective tool for the detoxification of xenobiotics.[13] Previously, two bacterial strains that are able to cometabolize buprofezin have been reported.[14,15]

In this work, we isolated and characterized a buprofezin-degrading strain of *Rhodococcus* sp., referred to as YL-1, that could utilize buprofezin as the sole carbon and nitrogen source for growth. A novel biodegradation pathway of buprofezin was proposed, and a pilot-scale bioremediation experiment using buprofezin-contaminated soil and strain YL-1 was conducted.

MATERIALS AND METHODS

Chemicals and Media. Buprofezin (99% purity), 2-isothiocyanato-2-methyl-propane (99% purity), 2-isothiocyanato-propane (97% purity), and high-performance liquid chromatography (HPLC) gradient-grade methanol were purchased from Sigma-Aldrich (St. Louis, MO). Analytical-grade dichloromethane, acetone, and ethyl acetate were purchased from the Shanghai Chemical Reagent Co., Ltd. (China). All other chemicals and reagents were of analytical grade and are available commercially.

Luria−Bertani (LB) medium contained 10.0 g L^{-1} tryptone, 5.0 g L^{-1} yeast extract, and 5.0 g L^{-1} NaCl, pH 7.0. Mineral salts medium (MM) contained 1.5 g L^{-1} K_2HPO_4, 0.5 g L^{-1} KH_2PO_4, 1.0 g L^{-1} NH_4NO_3, 0.2 g L^{-1} $MgSO_4{\cdot}7H_2O$, 1.0 g L^{-1} NaCl, and a 10 mL L^{-1} trace element solution,[16] pH 7.0. MM-1 medium contained 1.5 g L^{-1} K_2HPO_4, 0.5 g L^{-1} KH_2PO_4, 0.2 g L^{-1} $MgSO_4{\cdot}7H_2O$, 1.0 g L^{-1} NaCl, and a 10 mL L^{-1} trace element solution, pH 7.0. Buprofezin (50 mg L^{-1}) was added into MM or MM-1 to make BMM or BMM-1 media, respectively. Solid media plates were prepared by adding 15 g L^{-1} agar.

Isolation and Identification of Buprofezin-Degrading Bacteria. A soil sample was collected from the top 0−20 cm of a rice field in Yixing, Jiangsu Province, China. The chemical properties of soil were analyzed by the Institute of Soil Science, Chinese Academy of Sciences. The pH value of the soil sample was 6.49. The organic

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matter content was 39.6 g kg^{-1}, and the total organic carbon and organic nitrogen contents were 23.8 and 3.1 g kg^{-1}, respectively; the available K content was 146 mg kg^{-1}. Approximately 5 g of the soil sample was added to a 250 mL flask containing 100 mL of BMM medium with buprofezin as the sole carbon source and incubated at 30 °C on a rotary shaker at 160 rpm. Approximately 5 mL of each enrichment culture was transferred to 100 mL of fresh BMM every 7 days. The final dilutions of the sequential enrichments were plated on BMM agar plates, and different bacterial colonies were picked, purified, and tested for their buprofezin-degrading ability.[17]

The isolate was identified on the basis of its morphological, physiological, and biochemical properties[18] (with reference to *Bergey's Manual of Determinative Bacteriology*) and its 16S rRNA gene sequence. The cell morphology was analyzed by light microscopy (BH-2, Olympus, Japan) and transmission electron microscopy (TEM) (H-7650, Hitachi High-Technologies Corp., Japan) using cells from an exponentially growing culture. The genomic DNA of this strain was extracted by high-salt precipitation.[19] Two PCR primers, 20F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1500R (5′-GGTTACCTTGTTACGACTT-3′), were designed to amplify the 16S rRNA gene.[20] The 1482 bp 16S rDNA sequence was compared to sequences in GenBank using Blast.[21] Alignment of 16S rRNA gene sequences from GenBank was performed using Clustal X 1.8.3 with the default settings.[22] For further phylogenetic analysis, MEGA version 4.0 was used.[29] Each distance was calculated using the Kimura two-parameter distance model. Unrooted trees were built using the neighbor-joining method.[23] The data set was bootstrapped 1000 times.

Growth and Degradation Experiments Using Buprofezin. Strain YL-1 was precultured in 50 mL of LB media for 24 h. Cells were harvested by centrifugation at 5000*g* for 5 min at room temperature and washed twice with sterilized water. The optical density at 600 nm (OD_{600}) was adjusted to 2.0. For all experiments, unless otherwise stated, the cells were inoculated at 5% (v/v) into a 150 mL flask containing 50 mL of BMM-1. All of the cultures were incubated at 30 °C and 160 rpm on a rotary shaker. Samples were collected from the cultures at an interval of 8 h, and the amount of residual buprofezin was determined by HPLC. Bacterial growth was monitored by counting the colony forming units (cfu/mL) of serial dilutions. Each treatment was performed three times; uninoculated cultures and cultures without substrate incubated under the same conditions served as controls.

To determine the effect of the temperature on degradation, the cultures were incubated at 20, 25, 30, and 37 °C. To investigate the effect of the initial pH value on degradation, the pH value of the medium was adjusted to 4.0, 5.0, 6.0, 7.0, 8.0, and 9.0. To study the effect of the initial concentration of buprofezin on degradation, MM medium containing 30, 50, 100, or 200 mg L^{-1} buprofezin was used.

Substrate Range. To determine its ability to degrade nitrogen-containing pesticides, strain YL-1 was inoculated into MM-1 medium supplemented with 50 mg L^{-1} pesticide (imazethapyr, metsulfuron, isoproturon, thifensulfuron, benzyl ethyl, nicosulfuron, metribuzin, imidacloprid, and carbendazim). The degradation of these pesticides was qualitatively analyzed by HPLC.

Assays of Catechol 1,2- and 2,3-Dioxygenase Activities. The activities of catechol 1,2-dioxygenase (CD-1,2) and catechol 2,3-dioxygenase (CD-2,3) were assayed by the method of Liu et al.[24] *Rhodococcus* sp. YL-1 was grown at 30 °C in MM, BMM, or MM medium supplemented with glucose (50 mg L^{-1}). The cells were harvested during the period of maximum growth by centrifugation at 5000*g*, washed three times with 20 mM phosphate buffer (pH 7.0), and then resuspended in the same buffer to a cell density (OD600 nm) of 6.0 (resting cells). The resuspended cells were disrupted by sonication (Auto Science, UH-650B ultrasonic processor, 30% intensity) for 10 min and centrifuged at 10000*g* for 20 min at 4 °C. The clear supernatant was used for determining the activities of catechol dioxygenases. The protein concentration was determined according to the method of Bradford[25] using bovine serum albumin as the standard. One unit of catechol oxygenase was defined as the amount of protein required to catalyze the oxidation of 1 μmol of catechol per minute.

Degradation of Buprofezin in Soil. A soil sample that had never been exposed to buprofezin was collected from the top 0−20 cm from the foot of Zijin Mountain in Nanjing of Jiangsu Province, China. The pH value of the soil sample was 6.59. The samples were first sterilized by autoclaving at 121 °C for 60 min.[26] Then, approximately 200 g of fresh subsamples of soil and sterile soil was weighed, and the liquid form of buprofezin was mixed into the soil to obtain a final concentration of 10 mg kg^{-1} soil. Two sets of sterile and nonsterile soils were inoculated with strain YL-1 (10^8 cells g^{-1}). Another two soil samples inoculated with heat-killed YL-1 cells served as the control. The inoculum was thoroughly mixed into the soil under sterile conditions, and the soil moisture was adjusted to 35% (w/w) with sterile water. Each soil treatment was incubated at 30 °C in the dark. Every 5 days, 10 g of each soil sample was collected, and the concentration of buprofezin was determined. When strain YL-1 grows on BMM-1 agar (supplemented with 50 mg L^{-1} buprofezin) for several days, due to the degradation of buprofezin, a transparent ring will form around the colony (the solubility of buprofezin in water is only 9 mg L^{-1}) (Figure S1 in the Supporting Information). Few indigenous microorganisms from the soil could grow on BMM-1 agar containing buprofezin, and in these cases, no transparent ring was observed around the colonies. Therefore, the strain YL-1, which was reisolated from the soils, could be discriminated from the indigenous bacteria by formation of the transparent ring. The survival of strain YL-1 that was added to the soil was monitored using the formation of the transparent ring to identify YL-1 colonies. Experiments were repeated in triplicate.

Chemical Analysis. In liquid culture, buprofezin was extracted once with an equal volume of dichloromethane. The remaining aqueous phase was then acidified to pH 3.0 with 1 N HCl and extracted twice with an equal volume of ethyl acetate.[27] The extracts were pooled, dehydrated with anhydrous sodium sulfate, and then concentrated by revolving evaporation. The product was dissolved in a small amount (1 mL) of methanol. A portion of this extract was analyzed by HPLC.

Buprofezin extraction from soil was conducted according to the method described in a previous report.[28] Ten grams of each soil sample was extracted with 20 mL of ethanol:benzene solution (1:4, v/v), and the mixture was shaken for 2 h at 180 rpm on a rotary shaker. The eluate was collected and filtered through anhydrous sodium sulfate into an Erlenmeyer flask. The residues were extracted once again with 15 mL of ethanol:benzene solution (1:4, v/v) and shaken for 1.5 h. The remaining solution was dehydrated with anhydrous sodium sulfate. The filtrates were pooled and evaporated using a vacuum rotary evaporator at room temperature. Residual organic material was redissolved in 1 mL of methanol for HPLC analysis.

HPLC (600 controller, Rheodyne 7725i manual injector and 2487 Dual λ-Absorbance Detector; Waters Co., Milford, MA) was used in this study. Kromasil 100-5 C_{18} was used at the stationary phase in the separation column (25 cm length and 4.6 mm internal diameter). The mobile phase was methanol:water (7:3, v/v) containing 50 mM ammonium acetate, and the flow rate was 1.0 mL min^{-1}.[29] Buprofezin was analyzed at 245 nm using a liquid chromatography−mass spectrometry system (Agilent 1100 HPLC, Waters Q-TOF Micro) equipped with an electrospray ionization source and operated in the positive polarity mode. The electrospray and ring lens voltages were 3.0 kV and 20 V, respectively. The nebulizing and drying gas flow rates were 400 and 50 L/h, respectively. The nebulizing chamber and ion source temperatures were 200 and 100 °C, respectively.

To identify the metabolites produced during buprofezin degradation, GC−MS analyses were performed on a Thermo Trace DSQ mass spectrometer, under the following conditions. Helium was used as a carrier gas at a flow rate of 1.2 mL min^{-1}. Gas chromatography was conducted using a RTX-5 MS column (15 m × 0.25 mm × 0.25 mm, Restek Corp., United States). The column temperature was increased from 50 (1.5 min hold) to 200 °C (1 min hold) at 20 °C min^{-1} and then from 200 to 280 °C (20 min hold) at 40 °C min^{-1}. The injector temperature was set at 220 °C with a split ratio of 20:1. The interface temperature and ion source temperature were both set to 280 °C, and the mass was scanned in the range from 50 m/z to 650 m/z. The

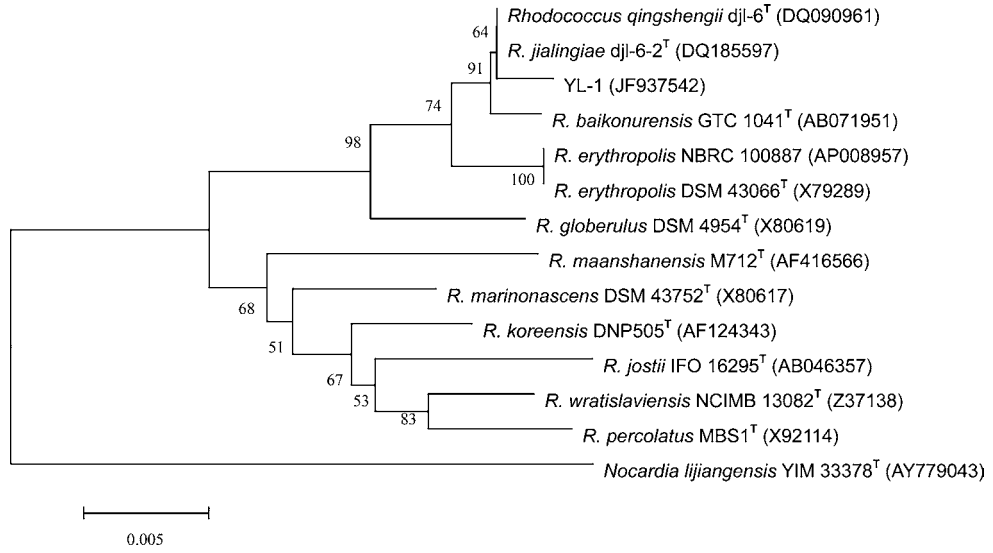

Figure 1. Phylogenetic analysis of strain YL-1 and related species using the neighbor-joining approach.

column outlet was inserted directly into the electron ionization source block, operating at 70 eV.[30,31]

RESULTS AND DISCUSSION

Strain Isolation and Identification. Buprofezin has been widely used in the control of rice planthoppers for many years in Jiangsu Province, China. Buprofezin residues were frequently found in the soil; therefore, it is probable that several bacteria have adapted to this buprofezin-contaminated environment. Using buprofezin as the sole carbon source, several buprofezin-degrading bacterial strains were isolated, and strain YL-1 was selected for further study due to its superior degrading ability. YL-1 showed elementary branching in the early growth phase, and most of the bacteria were short rods or cocci during the stationary phase. The cells are Gram-positive, aerobic, and nonmotile. The colonies on the LB plates are opaque, orange, moist, and convex with irregular edges. The optimal pH and temperature for the growth of strain YL-1 were 7.0 and 30 °C, respectively. This strain tested negative in the oxidase, starch hydrolysis, methyl red, and nitrate reductase tests and tested positive in the catalase, urease, and Voges–Proskauer tests. The phylogenetic tree based on the 16S rRNA gene sequence of strain YL-1 (accession no. JF937542) was shown in Figure 1. The 16S rRNA gene sequence of strain YL-1 showed 99.7% similarity to those of *Rhodococcus qingshengii* strain djl-6^T and *Rhodococcus jialingiae* strain djl-6-2^T. On the basis of the above phenotypic characteristics and the phylogenetic analysis, strain YL-1 was primarily identified as a species of *Rhodococcus*.

Degradation of Buprofezin by Strain YL-1. The kinetics of buprofezin degradation and strain YL-1 growth were investigated simultaneously in BMM-1 medium, to which buprofezin (99% purity) was added as the sole carbon and nitrogen source. Approximately 92% of the buprofezin was degraded after 48 h, and at this time, change in the number of strain YL-1 cells increased to its maximum value (Figure 2). The N and C sources that supported the growth of strain YL-1 could come from the MM-1 medium, buprofezin, or impurities in the buprofezin. First, in the control test, no obvious change in the number of cells was observed in the MM-1 medium without buprofezin, indicating that the trace N and C sources in the MM-1 medium could not support the growth of strain YL-1. Second, the buprofezin that was added had a purity of 99%,

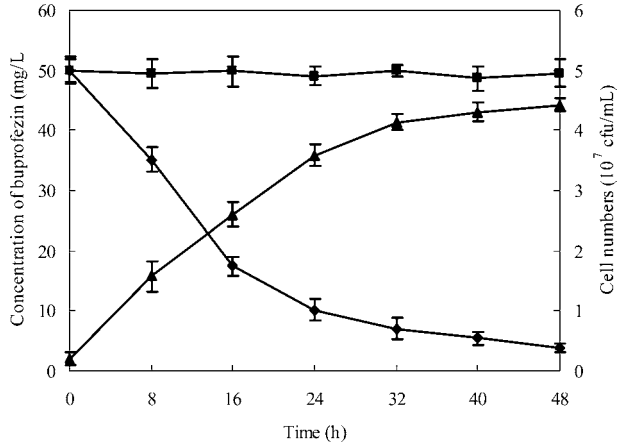

Figure 2. Degradation dynamics of buprofezin by *Rhodococcus* sp. strain YL-1. Error bars represent the standard error of three replicates. Uninoculated medium, ■; buprofezin concentration, ♦; and cell density, ▲.

and no other chemicals could be detected in the buprofezin using GC and HPLC analyses. The amounts of N and C in the impurities (1%) could not support the significant change in cell accumulation that was observed; therefore, we conclude that the strain YL-1 could grow on buprofezin as the sole carbon and nitrogen source. However, this strain failed to degrade other nitrogen-containing pesticides, including imazethapyr, metsulfuron, isoproturon, thifensulfuron, benzyl ethyl, nicosulfuron, metribuzin, imidacloprid, and carbendazim.

The degradation of buprofezin by strain YL-1 occurred over a wide range of temperatures and pH values. The strain could effectively degrade buprofezin at temperatures ranging from 25 to 30 °C (Figure S2 in the Supporting Information) and at pH values from 5.0 to 9.0 (Figure S3 in the Supporting Information). The optimum degradation temperature and pH were 30 °C and 7.0, respectively. Further study showed that strain YL-1 was able to degrade buprofezin at initial concentrations of 100 and 200 mg L^{-1}, and 52.4 and 32.8%, respectively, of the initial buprofezin was degraded in 48 h (Figure S4 in the Supporting Information).

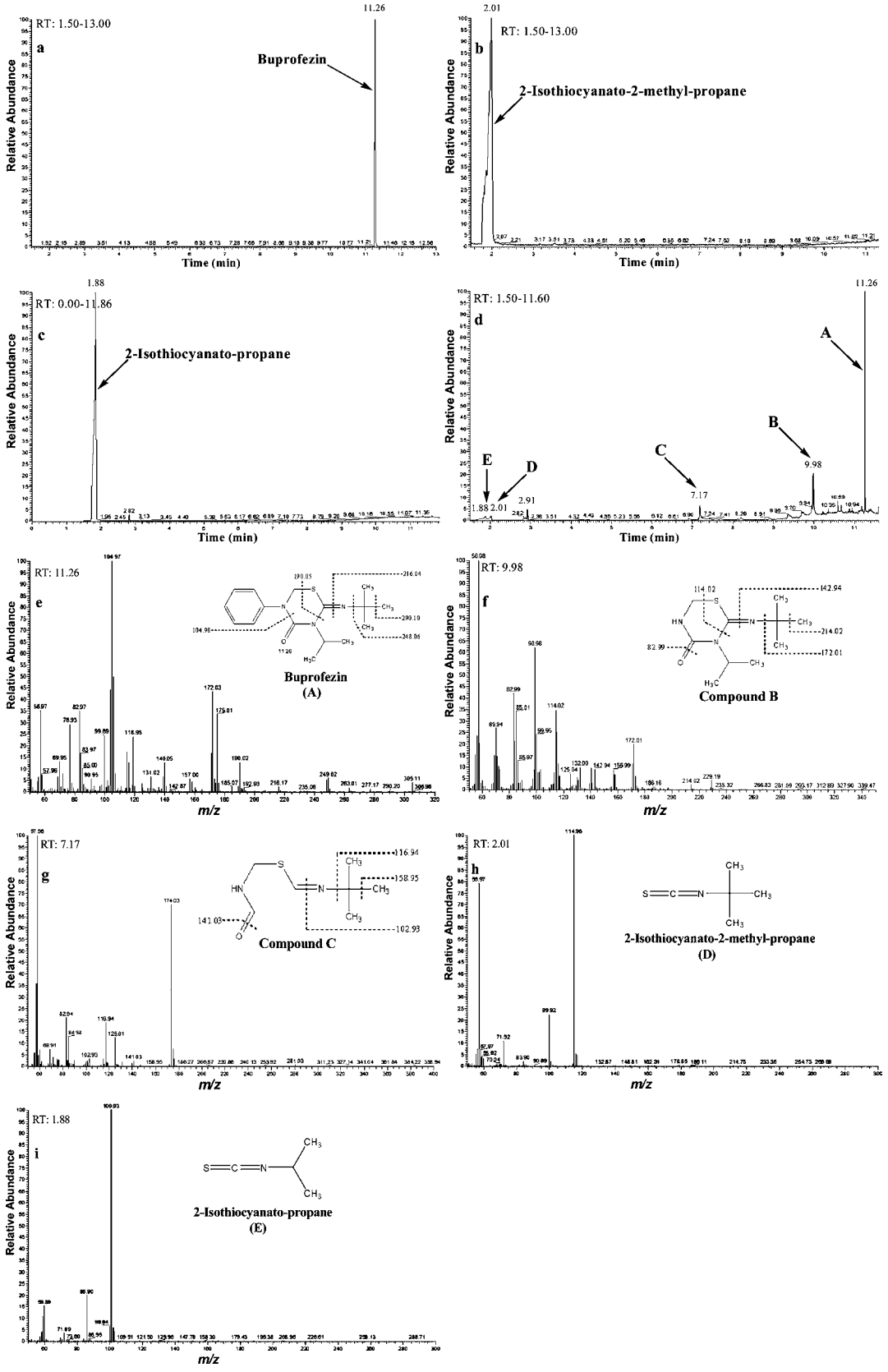

Figure 3. GC−MS chromatograms (a−d) and the characteristic fragment ions (e−i) of buprofezin, 2-isothiocyanato-2-methyl-propane, 2-isothiocyanato-propane, and the metabolites. Compounds corresponding to peaks with retention times of 11.26, 9.98, 7.17, 2.01, and 1.88 min in the GC−MS chromatograms (d) are marked with letters A, B, C, D, and E, respectively.

Identification of the Metabolites during Buprofezin Degradation. The products of the degradation of buprofezin by strain YL-1 and the authentic chemicals were analyzed by GC−MS. The GC−MS chromatograms for the standards of

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buprofezin, 2-isothiocyanato-2-methyl-propane, and 2-isothiocyanato-propane are shown in Figure 3a−c, respectively. The GC−MS chromatogram of the metabolites produced during buprofezin degradation is shown in Figure 3d. The compounds with retention times (RT) of 11.26, 9.98, 7.17, 2.01, and 1.88 min were designated compounds A, B, C, D, and E, respectively (Table 1).

Table 1. Buprofezin and Its Metabolites Identified by GC−MS

compd	chemical name	RT (min)	characteristic ions in GC−MS (m/z)
A	buprofezin	11.26	104.97, 118.95, 131.02, 140.05 157.00, 172.03, 190.02, 216.17, 248.02, 290.20, 305.11
B	2-*tert*-butylimino-3-isopropyl-1, 3,5-thiadiazinan-4-one	9.98	56.98, 82.99, 98.98, 114.02, 125.04, 142.94, 156.99, 172.01, 214.02, 229.19
C	*N*-*tert*-butyl-thioformimidic acid formylaminomethyl ester	7.17	57.96, 84.98, 102.93, 116.94, 141.03, 158.95, 174.03
D	2-isothiocyanato-2-methyl-propane	2.01	56.97, 71.92, 99.92, 114.95
E	2-isothiocyanato-propane	1.88	59.89, 71.89, 85.90, 100.93

Compound A had the same RT as the buprofezin standard (RT = 11.26 min). The molecular ion (M^+) peak of compound A was 305 m/z with characteristic fragment ions at 290.20 m/z (M^+-CH_3), 248.02 m/z ($M^+-C(CH_3)_3$), 216.17 m/z ($M^+-CN(CH_3)_3-H_2O$), 190.02 m/z ($M^+-C(CH_3)_3-CN-S$), 172.03 m/z ($M^+-C(CH_3)_3-CN-S-H_2O$), 157.00 m/z ($M^+-C(CH_3)_3-CN-S-CH_3-H_2O$), 131.02 m/z ($M^+-C(CH_3)_3-CN-S-H_2O-CH(CH_2)_2$), 118.95 m/z ($M^+-CN(C(CH_3)_3)-N(CH(CH_3)_2)-CH_2S$), and 104.97 m/z ($M^+-CN(C(CH_3)_3)-N(CH(CH_3)_2)-S-CO$). These mass spectral data were identical to those for the buprofezin standard and were easily matched to buprofezin using the standard GC−MS library. Therefore, compound A was identified as buprofezin (Figure 3a,d,e).

Compounds D and E had the same RTs as the standards of 2-isothiocyanato-2-methyl-propane (RT = 2.01 min) and 2-isothiocyanato-propane (RT = 1.88 min), respectively. The mass spectra of these two metabolites were identical to the spectra of their standards and were also easily matched with the correct compounds using the standard library. Thus, it could be confirmed that compounds D and E were 2-isothiocyanato-2-methyl-propane (Figure 3b,d,h) and 2-isothiocyanato-propane (Figure 3c,d,i), respectively.

We could not obtain the authentic chemicals for compounds B and C, and they were identified using GC−MS and LC−MS analyses. The M^+ peak of compound B was 229 m/z, and the characteristic fragment ions were 214.02 m/z (M^+-CH_3), 172.01 m/z ($M^+-C(CH_3)_3$), 156.99 m/z ($M^+-C(CH_3)_3-CH_3$), 142.94 m/z ($M^+-CN(CH_3)_3-CH_3$), 125.04 m/z ($M^+-CN(CH_3)_3-H_2O-CH_3$), 114.02 m/z ($M^+-CN(C(CH_3)_3)-S$), 98.98 m/z ($M^+-CN(C(CH_3)_3)-S-CH_3$), 82.99 m/z ($M^+-CN(CH_3)_3-N(CH(CH_3)_2)-H_2O$), and 56.98 m/z ($M^+-N(CH(CH_3)_2)-CO-NH-CH_2S-C$) (Figure 3f). Compound C had a M^+ peak of 174 m/z, and its characteristic fragment ion peaks were 158.95 m/z (M^+-CH_3), 141.03 m/z ($M^+-CH_3-H_2O$), 116.94 m/z ($M^+-C(CH_3)_3$), 102.93 m/z ($M^+-CN(CH_3)_3$), 84.98 m/z ($M^+-CN(CH_3)_3-H_2O$), and 57.96 m/z ($M^+-C(CH_3)_3-CHO-NH-CH_2$) (Figure 3g). Their mass spectra could not be matched with any spectra in the standard GC−MS library, but on the basis of both their molecular ions and fragment ions, they were tentatively proposed to be 2-*tert*-butylimino-3-isopropyl-1,3,5-thiadiazinan-4-one (B) and *N*-*tert*-butyl-thioformimidic acid formylaminomethyl ester (C). In addition, LC−MS analysis demonstrated the presence of 2-*tert*-butylimino-3-isopropyl-1,3,5-thiadiazinan-4-one (B), *N*-*tert*-butyl-thioformimidic acid formylaminomethyl ester (C), and buprofezin (A), results that are consistent with the corresponding GC−MS analysis (Figure S5 and Table S1 in the Supporting Information).

Activities of Catechol Dioxygenase. As shown in Table 2, a high level of CD-2,3 activity was induced due to buprofezin exposure, whereas CD-1,2 activity was not detected under any of the tested conditions, suggesting that CD-2,3 is involved in the biodegradation of buprofezin in strain YL-1. The expression of CD-2,3 is usually induced by catechol or its derivatives. Although catechol was not detected, compound B was identified as 2-*tert*-butylimino-3-isopropyl-1,3,5-thiadiazinan-4-one; therefore, we speculated that the benzenic ring was first removed from buprofezin, generating 2-*tert*-butylimino-3-isopropyl-1,3,5-thiadiazinan-4-one and catechol, the latter of which then induced the expression of CD-2,3.

Table 2. Specific Activities of Catechol Dioxygenases of *Rhodococcus* sp. YL-1 Cultured in MM with Different Carbon Sources

growth substrates	catechol 1,2-dioxygenase (CD-1,2) (U/mg protein)	catechol 2,3-dioxygenase (CD-2,3) (U/mg protein)
buprofezin	0	0.41 ± 0.06
a	0	0
glucose	0	0

[a]No substrate was added.

On the basis of above results, we propose a transformation pathway for buprofezin in *Rhodococcus* sp. YL-1 (Figure 4); this proposed pathway is completely different from the cometabolizing pathway in strain DFS35-4.[14] The benzenic ring is first removed from buprofezin to generate catechol and 2-*tert*-butylimino-3-isopropyl-1,3,5-thiadiazinan-4-one, and the latter is then transformed into *N*-*tert*-butyl-thioformimidic acid formylaminomethyl ester, followed by conversion into 2-isothiocyanato-2-methyl-propane; 2-isothiocyanato-2-methyl-propane is subsequently degraded into 2-isothiocyanato-propane through partial C-dealkylation. The catechol undergoes *meta*-cleavage (CD-2,3) and further metabolism.

Biodegradation of Buprofezin in Soil. The inoculation of soil with strain YL-1 significantly accelerated the degradation of buprofezin in the soil. After 25 days of incubation, only approximately 6% of buprofezin was degraded in noninoculated sterilized soil. In contrast, in the soil inoculated with strain YL-1, the degradation rate rose to 84% under the same conditions (Figure 5), representing a significant increase in the buprofezin degradation efficiency. In the inoculated and uninoculated fresh soil samples, the degradation rates of buprofezin were 85.3 and 12.1%, respectively, after 25 days. When added to the soil, strain YL-1 could be detected after 25 d (1.9×10^7 cfu g^{-1}), showing that strain YL-1 could sustain itself in the natural environment for a long period. These results indicate the potential use of strain YL-1 in the bioremediation of buprofezin-contaminated soil.

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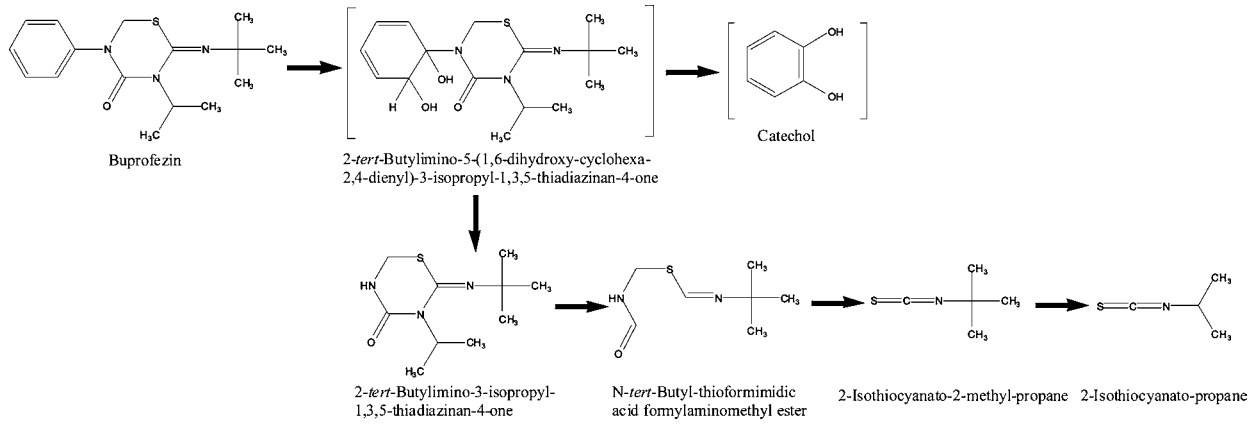

Figure 4. Proposed degradation pathway of buprofezin by strain YL-1 based on the presumed metabolites.

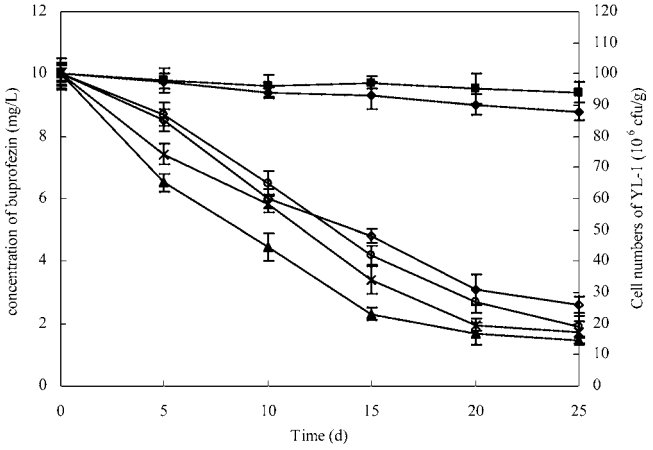

Figure 5. Degradation of buprofezin in varied soils. Error bars represent the standard error of three replicates. Sterilized soil without inoculation, ■; nonsterilized soil without inoculation, ♦; sterilized soil inoculated with strain YL-1, ×; number of strain YL-1 cells, ◊; nonsterilized soil inoculated with strain YL-1, ▲; and number of strain YL-1 cells, ○.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Tables of compounds A−C identified by LC−MS and chemical structures of different nitrogen-containing pesticides. Figures of transparent ring formed around a strain YL-1 colony on BMM-1 agar; effects of temperature, initial pH, and concentration on the degradation of buprofezin; and LC−MS mass data of compounds A−C. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel/Fax: +86-025-84396314. E-mail: yanxin@njau.edu.cn.

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Notes

The authors declare no competing financial interest.

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