ISSN 1001-0742
CN 11-2629/X

2012

Volume 24
Number 8

JOURNAL OF ENVIRONMENTAL SCIENCES

ELSEVIER
Science Press

Sponsored by
Research Center for Eco-Environmental Sciences
Chinese Academy of Sciences

JOURNAL OF ENVIRONMENTAL SCIENCES

(http://www.jesc.ac.cn)

Aims and scope

Journal of Environmental Sciences is an international academic journal supervised by Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences. The journal publishes original, peer-reviewed innovative research and valuable findings in environmental sciences. The types of articles published are research article, critical review, rapid communications, and special issues.

The scope of the journal embraces the treatment processes for natural groundwater, municipal, agricultural and industrial water and wastewaters; physical and chemical methods for limitation of pollutants emission into the atmospheric environment; chemical and biological and phytoremediation of contaminated soil; fate and transport of pollutants in environments; toxicological effects of terrorist chemical release on the natural environment and human health; development of environmental catalysts and materials.

For subscription to electronic edition

Elsevier is responsible for subscription of the journal. Please subscribe to the journal via http://www.elsevier.com/locate/jes.

For subscription to print edition

China: Please contact the customer service, Science Press, 16 Donghuangchenggen North Street, Beijing 100717, China. Tel: +86-10-64017032; E-mail: journal@mail.sciencep.com, or the local post office throughout China (domestic postcode: 2-580).

Outside China: Please order the journal from the Elsevier Customer Service Department at the Regional Sales Office nearest you.

Submission declaration

Submission of an article implies that the work described has not been published previously (except in the form of an abstract or as part of a published lecture or academic thesis), that it is not under consideration for publication elsewhere. The submission should be approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out. If the manuscript accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright-holder.

Submission declaration

Submission of the work described has not been published previously (except in the form of an abstract or as part of a published lecture or academic thesis), that it is not under consideration for publication elsewhere. The publication should be approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out. If the manuscript accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright-holder.

Editorial

Authors should submit manuscript online at http://www.jesc.ac.cn. In case of queries, please contact editorial office, Tel: +86-10-62920553, E-mail: jesc@263.net, jesc@rcees.ac.cn. Instruction to authors is available at http://www.jesc.ac.cn.

Copyright

ISSN 1001–0742 **Journal of Environmental Sciences** Vol. 24 No. 8 2012

CONTENTS

Serial parameter: CN 11-2629/X*1989*m*184*en*P*24*2012-8

Available online at www.sciencedirect.com
ScienceDirect
Journal of Environmental Sciences 2012, 24(8) 1492–1497
JOURNAL OF ENVIRONMENTAL SCIENCES
ISSN 1001-0742
CN 11-2629/X
www.jesc.ac.cn

Augmentation of tribenuron methyl removal from polluted soil with *Bacillus* sp. strain BS2 and indigenous earthworms

Qiang Tang[1,**], Zhiping Zhao[1,**], Yajun Liu[1,**], Nanxi Wang[1,2], Baojun Wang[1], Yanan Wang[3], Ningyi Zhou[4], Shuangjiang Liu[1,*]

1. State Key Laboratory of Microbial Resources, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China. E-mail: tqan10000@163.com
2. College of Life Science, University of Science and Technology, Hefei 230026, China
3. Key Laboratory of Microbial Engineering at the Institute of Biology, Henan Academy of Sciences, Zhengzhou 450008, China
4. Key Laboratory of Agricultural and Environmental Microbiology, Wuhan Institute of Virology, Chinese Academy of Sciences, 44 Xiao-Hong-Shan, Wuhan 430071, China

Received 16 October 2011; revised 14 November 2011; accepted 30 November 2011

Abstract

Tribenuron methyl (TBM) is a member of the sulfonylurea herbicide family and is widely used worldwide. In this study, TBM-degrading bacteria were enriched with TBM as potential carbon, nitrogen or sulfur source, and 44 bacterial isolates were obtained. These isolates were phylogenetically diverse, and were grouped into 25 operational taxonomic units and 14 currently known genera. Three representatives, *Bacillus* sp. strain BS2, *Microbacterium* sp. strain BS3, and *Cellulosimicrobium* sp. strain BS11, were selected, and their growth and TBM removal from culture broth were investigated. In addition, indigenous earthworms were collected and applied to augment TBM degradation in lab-scale soil column experiments. Results demonstrated that *Bacillus* sp. strain BS2 and earthworms significantly increased TBM removal during soil column experiments.

Key words: biodegradation of tribenuron methyl; *Bacillus*; *Microbacterium*; *Cellulosimicrobium*; earthworm

DOI: 10.1016/S1001-0742(11)60947-9

Introduction

Tribenuron methyl (TBM), chemically 2-[4-methoxy-6-methyl-1,3,5-triazin-2-yl(methyl)carbamoylsulfamoyl] benzoic acid, is a member of the sulfonylurea herbicide family and it kills weeds by inhibiting synthesis of the branched chain amino acids (valine and isoleucine), hence stopping cell division and weed growth. TBM is widely used worldwide as well as in China. Although sulfonylurea herbicides are considered to be highly efficient at low dosage, their phytotoxicity to sensitive crops has resulted in crop yield reduction in agricultural rotation systems (Beyer et al., 1987; Anderson et al., 2001; Ye et al., 2003). For example, the application of TBM in wheat fields in Henan province led to severely reduced rotated-crop's growth and production, e.g., corn and cotton, due to the phytotoxicity of residual TBM in soil. It is also known that the fate of sulfonylurea compounds in soil is determined by many factors, including physical-chemical factors such as soil acidity, temperature and moisture content, as well as soil fauna and their biological activity (Walker et al., 1993; Ravelli et al., 1997; Wang et al., 2010). The half-life of TBM in soil is generally considered to be 1–7 days, however, investigation has shown that only 25% of TBM (initial TBM concentration of 20 mg/kg of dry soil) was mineralized after 126 days in sandy soil (Anderson et al., 2001).

In the attempt to eliminate phytotoxicity to rotated crops, fast and effective methods are desired to remove residual sulfonylurea herbicides from soil, including TBM. Some bacterial strains that are able to degrade sulfonylurea herbicides have been reported. It has been reported that *Pseudomonas* sp. strain LW3 was able to degrade chlorimuron ethyl (Ma et al., 2009), and *Bacillus* sp. strain L1 was able to degrade bensulfuron methyl (Lin et al., 2010). The effectiveness of bacterial degraders in the removal of sulfonylurea herbicides was evaluated with soil compartments (Huang et al., 2007; Si et al., 2005).

Earthworms were recognized as soil engineers (Scheu, 1987; Jones et al., 1994), and they could change the physical-chemical factors of soil, such as moisture, gas exchange of soils and nutrient availability (Lavelle, 1988). The soils that were influenced by earthworms were characterized to have higher microbial activities. This suggests that earthworms could stimulate biodegradation of herbicides through activation of special microbial degraders (Drake and Horn, 2007). These previous studies have proved the effectiveness of earthworms in bioremediation

* Corresponding author. E-mail: liusj@mail.im.ac.cn. ** The authors contribute equally to this work.

(Schaefer and Filser, 2007; Liu et al., 2011).

In this study, bacterial strains that are able to degrade TBM were isolated and screened. Their biodegradation ability was tested in liquid cultures. Application of TBM degraders and indigenous earthworms in the removal of TBM from soil was investigated in lab-scale soil columns.

1 Materials and methods

1.1 Samples, media, enrichment and isolation of TBM degrading bacteria

Soil samples were collected from a pesticide processing factory located in a suburb of Hengshui City, Hebei Province, China. A complete medium composed of the following components was designed: KH_2PO_4 (0.5 g/L), $Na_2HPO_4{\cdot}12H_2O$ (1.0 g/L), NH_4Cl (1.1 g/L), $MgSO_4{\cdot}7H_2O$ (0.2 g/L), glucose (0.8 g/L), trace elements solution (2 mL/L) (Kaminski et al., 1983), yeast extract (0.03 g/L), and 50 mg/L of TBM. Four media were developed from the complete medium: Medium 1 (M1) was modified to enrich and isolate TBM-degrading bacteria that take TBM as a carbon source, thus the glucose in the complete medium was deleted. Medium 2 (M2) was modified to enrich and isolate TBM-degrading bacteria that take TBM as a nitrogen source, thus the NH_4Cl in the complete medium was deleted. Medium 3 (M3) was modified to enrich and isolate TBM-degrading bacteria that take TBM as a sulfur source, thus the $MgSO_4{\cdot}7H_2O$ in the complete medium was replaced by $MgCl_2$. Medium 4 (M4) was designed to observe whether short chain fatty acids would stimulate the growth of TBM-degrading bacteria, thus the glucose was deleted from the complete medium and a mixed solution of short chain fatty acids (containing sodium succinate, sodium acetate, sodium citrate, sodium benzoate and sodium lactate, each at 200 mg/L) was included. All media were adjusted to pH 7.3, and were autoclaved at 115°C for 20 min. TBM stock solution was separately sterilized by filtration through 0.2 μm bacteria-retentive filters and aseptically added into the autoclaved medium.

To start the enrichments, about 3.0 g of the soil samples were added to 250 mL-Erlenmeyer flasks containing 100 mL media broth of M1, M2, M3, or M4, and were incubated at 30°C in the dark and shaken at 150 r/min. About 5 mL of enrichment culture was weekly transferred into 100 mL freshly prepared medium. The enrichments showing efficient degradation of TBM were spread onto agar plates. Microbial colonies appeared on the plates after 2-day incubation at 30°C and bacterial strains were isolated by repeated streaking on fresh agar plates.

1.2 Cloning and sequencing of 16S rRNA gene and phylogeny of bacterial population and isolates

The 16S rRNA genes of isolates were amplified by polymerase chain reaction (PCR) with the universal primer pair of 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′). The conditions for PCR were as follows: 5 min of denaturation at 95°C followed by 30 cycles of 94°C for 1 min, 55°C for 1 min, 72°C for 1.5 min and a final extension at 72°C for 10 min. PCR products were visualized by agarose gel electrophoresis, and sequenced (BGI, Beijing, China). All the obtained 16S rRNA gene sequences were checked for chimeras and analyzed using BLASTn program, and phylogenetic analysis was performed with Mega 4.0.

1.3 Evaluation of bacterial degradation of TBM in cultural broth and in soil column

All isolates were evaluated for their ability to degrade TBM in the above mentioned media (M1, M2, M3, or M4). The experiments were conducted with 250-mL flasks containing 100 mL medium and 50 mg/L of TBM, and were incubated on a rotary shaker at 30°C and 150 r/min. Samples were collected at regular time intervals, cell growth and TBM degradation were determined.

Bacillus sp. strain BS2 was selected for soil column experiments. Soil column experiments were carried out in glass beakers (1 L, 10 cm diameter × 14 cm height). A large quantity of soil was removed from farmland in Linying County, Henan Province, and was transferred and stored at 4°C in the dark until use. Before use, the soil was ground and screened through 5 mm sieve. The soil sample of 1 kg was placed into a glass beaker. The soil was adjusted and maintained to a final moisture content of 25% (by weight) with sterile deionized water, and final TBM concentrations of 10 or 15 mg/kg dry soil.

The TBM-degrading *Bacillus* sp. strain BS2 was cultured in LB medium overnight, and cells were harvested by centrifugation at a speed of 5000 r/min for 10 min (BECKMAN COULTER, China). The pellet was washed twice with sterile deionized water to avoid residue nutrient into the soil. Cells were finally suspended with sterile water and counted with a blood cell counter. Cell density in soil was about 3×10^7 cells/g dry soil. The soil columns were kept in the dark at room temperature (around 25°C). In order to monitor the TBM degradation rates, 2 g soil in duplicate was sampled from the surface of each column and used for HPLC analysis.

1.4 Soil column experiments on TBM degradation with earthworms

The earthworms at a length of 10–15 cm were collected from the farmland in Linying County, Henan Province, and were chosen to perform the experiments. The soils used in the experiments was treated the same as described in Section 1.3, except that 2% (*W*/*W*) of ground fresh grass was added into the soil columns to feed the earthworms. The biomass of earthworms in each column was around 6 g/kg dry soil, which was about the *in situ* earthworm biomass.

1.5 Extraction of TBM and analysis by HPLC method

Sterile deionized water was adjusted to pH 8.0 and used to recover TBM from the soils. The recovery rate was tested. The TBM concentration was determined with an HPLC system (Agilent 1200 series, USA) equipped with an extend C_{18} column (4.6 mm × 250 mm × 5 μm) (Agilent,

USA). TBM was eluted with a mixture of acetonitrile and distilled water (50:50, *V*/*V*) at a speed of 1 mL/min. TBM molecules were detected at 230 nm with a diode array detector.

1.6 Data analysis and statistics

The data obtained from the soil column experiments was analyzed with Microsoft Office Excel 2007. The TBM degradation (amounts) either in fresh soil or in soil inoculated with *Bacillus* sp. strain BS2 or with earthworms was expressed as the average of all determinations at the specified time. The differences of TBM degradations between fresh soil columns and soil columns inoculated with *Bacillus* sp. strain BS2 or with earthworms were calculated according to the following methods: Difference (%) = [(sum of TBM degraded at each period in soil columns with bacteria or earthworms – sum of TBM degraded at each period in soil columns without bacteria or earthworms) ÷ (sum of TBM degraded at each period in soil columns without bacteria or earthworms)] × 100%.

1.7 Chemicals

TBM (95.8% purity) was purchased from Shanghai Pesticide Research Institute, China. Acetonitrile for high performance liquid chromatography (HPLC) analyses was HPLC grade (Fisher Scientific, UK). Other chemicals used were analytical grade.

2 Results

2.1 Isolation of TBM degraders and TBM degradation by diverse microbial isolates

TBM degraders were enriched from so-polluted soil samples for 50 days by 4 strategies (Section 1). In total, 44 bacterial strains were obtained, of which 11 were from M1 medium (TBM as carbon source), 14 strains were from M2 medium (TBM as nitrogen source), 12 from M3 medium (TBM as sulfur source), and 7 from M4 medium (a mixture of short chain fatty acids was supplied as potential stimulator for TBM-degraders). The ability of those bacterial strains to degrade TBM is listed in Table 1. Phylogenetic analysis based on 16S rRNA gene sequences revealed that the isolated strains were grouped into 25 operational taxonomic units (OTUs, all isolates that had 16S rRNA gene identity ⩾ 97% were grouped in one OTU). These 25 OTUs are phylogenetically associated with 14 currently known bacterial genera. *Bacillus* spp. were detected in all enrichments and were isolated with all 4 media, and they accounted for 31% of all bacterial isolates. The diversity of TBM-degrading bacteria was unexpectedly high, compared to the limited reports on bacterial TBM degraders.

2.2 Characterization of TBM degradation by strains BS2, BS3 and BS11

Based on the stability of TBM degradation and the pH of culture broth at the end of growth, 3 isolates were selected from the isolated TBM-degrading bacterial strains, namely *Bacillus* sp. strain BS2, *Microbacterium* sp. strain BS3 and *Cellulosimicrobiu*m sp. strain BS11, and they were subjected to further investigation. TBM degradation and cell growth are shown in Fig. 1. Cell growth of strain BS2 in the presence and absence of TBM was not noticeably different. It was observed also that the cell density of strain BS2 increased quickly within the first 30 hr and decreased quickly after that time, in the presence or absence of TBM (Fig. 1a). In contrast to strain BS2, the growth of strains BS3 and BS11 behaved differently. The presence of TBM slightly reduced the growth of strain BS3 (Fig. 1b). The growth of strain BS11 was opposite to strain BS2, and it reached higher cell density in the presence of TBM than without TBM (Fig. 1c). TBM removal by the 3 strains was obvious, and 30%–80% of TBM was removed after 170 hr incubation (Fig. 1).

2.3 *Bacillus* sp. strain BS2 stimulated TBM removal from soil column

The recovery rate of TBM from soil by sterile deionized water was (90 ± 4)%. In order to evaluate if the isolated TBM-degrading bacterial strains were potentially useful for bioremediation of polluted soil, *Bacillus* sp. strain BS2 was selected and applied for TBM-polluted soil remediation. As shown in Fig. 2, inoculation of strain BS2 in TBM-polluted soil apparently stimulated the removal of TBM. The TBM removal rate at the initial concentration of 10 mg TBM/kg dry soil was higher than that without

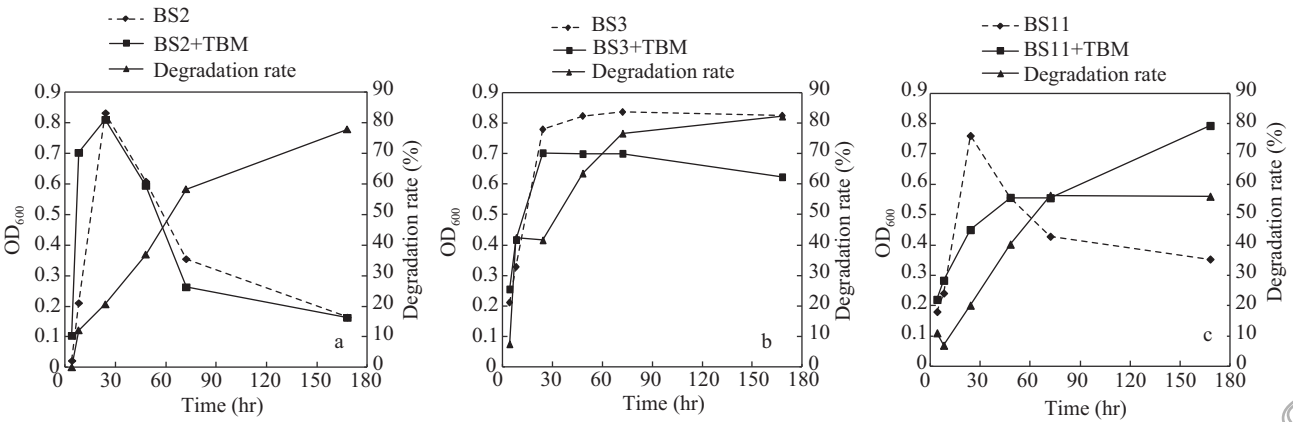

Fig. 1 Cell growth of *Bacillus* sp. strain BS2 (a), *Microbacterium* sp. BS3 (b) and *Cellulosimicrobium* sp. BS11 (c) in the presence and absence of TBM.

Table 1 Bacterial isolates, their closely phylogenetic relatives and their TBM degradation

Strain*	End pH	TBM removal	Phylogenetically closest related strains and 16S rRNA gene identities	
BN1	6.2	100%	*Kocuria rosea* CV1	100%
BN2	–	–	*Brevundimonas vesicularis* EQH12	100%
BN3	8.6	28.8%	*Rhodococcus globerulus* AZ1-15	100%
BN4	–	–	*Moraxella osloensis* PCWCW3	99%
BN5	8.5	0%	*Acinetobacter lwoffii* 0909CI4D_6	100%
BN6	–	–	*Bacillus megaterium* DSM319	100%
BN8	8.6	12.2%	*Bacillus megaterium* DSM319	100%
BN9	6.7	62.8%	*Bacillus anthracis* U13	100%
BN10	7.1	46.0%	*Bacillus vireti* A4	99%
BN11	7.8	9.8%	*Bacillus megaterium* DSM319	100%
BN12	8.3	25.2%	?	?
BN13	8.2	21.6%	?	?
BN14	8.2	18.2%	?	?
BN17	–	–	*Brevundimonas vesicularis* CE39	100%
BN22	–	–	*Acinetobacter lwoffii* BL Ac9	100%
BS1	7.7	29.8%	*Ochrobactrum tritici* pyd-1	100%
BS2	6.7	81.2%	*Bacillus arbutinivorans* BQN3L-02d	99%
BS3	6.3	100%	*Microbacterium lacticum* YC	99%
BS4	7.8	33%	*Brevundimonas diminuta* XW2a	99%
BS6	7.9	41.6%	*Ochrobactrum tritici* pyd-1	100%
BS7	7.5	36.2%	*Ochrobactrum tritici* pyd-1	100%
BS8	7.3	18.0%	*Ochrobactrum tritici* pyd-1	100%
BS9	6.9	78.0%	*Cellulosimicrobium cellulans* IBL10	100%
BS10	–	–	*Ochrobactrum tritici* pyd-1	100%
BS11	6.4	79.2%	*Cellulosimicrobium cellulans* IBL10	100%
BS12	7.5	35.0%	*Gordonia alkanivorans* DSM 44187	100%
BS13	8.1	29.2%	*Microbacterium oxydans* L2	99%
BC1	–	–	*Bacillus thioprans* BMP-1	100%
BC2	–	–	?	?
BC3	7.0	68.4%	*Bacillus arbutinivorans* BQN3L-02d	99%
BC4	–	–	*Bacillus thuringiensis* YBT-020	
BC10	7.0	40.4%	?	?
BC11	–	–	?	?
BC12	–	–	*Bacillus thioprans* BMP-1	100%
BC13	5.9	100%	*Bacillus firmus* strain D8	100%
BC14	6.0	100%	*Agromyces mediolanus* c70	99%
BC15	7.7	16.4%	*Ochrobactrum tritici* strain pyd-1	100%
BC16	7.0	38.8%	*Aminobacter niigataensis* DSM7050	97.5%
BHC1	7.6	9.0%	*Ochrobactrum tritici* pyd-1	100%
BHC2	7.9	17.4%	*Bacillus megaterium* 2EJ4	100%
BHC3	6.3	67.6%	*Sphingomonas adhaesiva* C2	99%
BHC4	7.8	26.2%	*Ochrobactrum tritici* pyd-1	100%
BHC6	7.9	23.8%	*Ochrobactrum tritici* pyd-1	100%
BHC7	7.4	32.8%	*Sphingobacterium multivorum* C2-30-2	99%
BHC8	8.2	17.8%	Comamonadaceae bacterium MPsc	99%

BN, BS, BC, and BHC stand for strains isolated from M1, M2, M3, and M4 media, respectively.
"–": these strains were lost during cultivation; "?": determination of the 16S rRNA genes was not successful.

strain BS2 (Fig. 2a). At higher initial TBM concentration of 15 mg TBM/kg dry soil, the removal rate with strain BS2 was also high (Fig. 2b). Statistically, the amounts of TBM eliminated from soil were 60.7% and 32.5% higher, respectively, at initial concentrations of 10 and 15 mg TBM/kg dry soil.

2.4 Bioaugmentation of TBM removal with indigenous earthworms

In this study, earthworms were collected from TBM-polluted soil and were cultivated in lab-scale soil columns that were supplemented with either 10 or 15 mg TBM/kg dry soil. The TBM removal rate with earthworms at the initial concentration of 10 mg TBM/kg dry soil was higher than that without earthworms during the period of day 3 to day 15 (Fig. 3a), although the removal rate became similar at extended cultivation. At the even higher initial TBM concentration of 15 mg TBM/kg dry soil, the removal rate was even more significantly higher (Fig. 3b). Statistically, the amounts of TBM eliminated from soil were 10.9% and 90.1% higher compared to that without earthworms, respectively, at initial concentrations of 10 and 15 mg TBM/kg dry soil.

3 Discussion

Due to the phytoxicity of sulfonylurea herbicides to rotated crops and the concerns of pollution of surface and ground waters, quick and efficient methods are needed to eliminate sulfonylurea herbicides from contaminated soils. For that purpose, many efforts have been made to obtain microbes that are able to degrade sulfonylurea herbicides. Several bacterial and fungal strains were reported to be able to degrade metsulfuron methyl, including *Pseudomonas* sp.

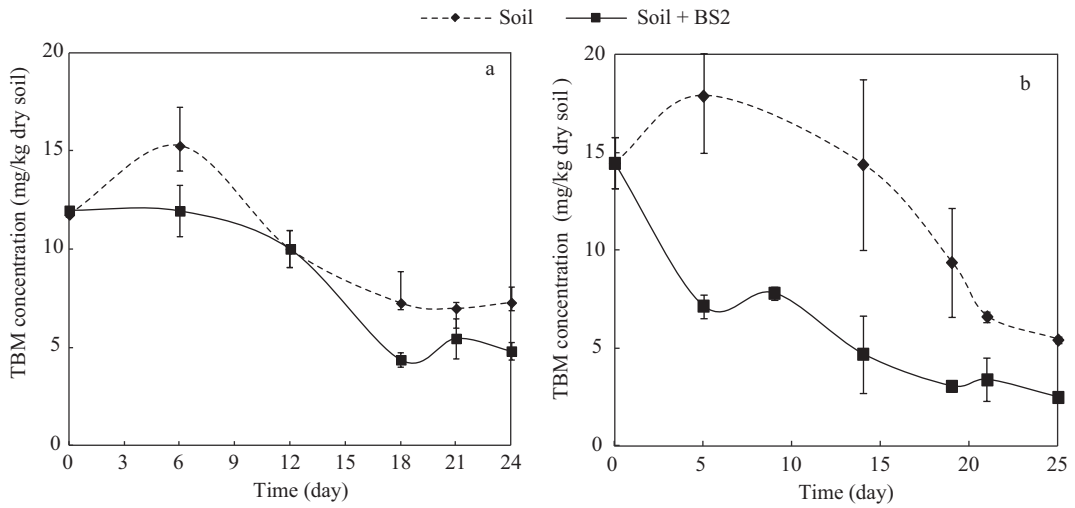

Fig. 2 *Bacillus* sp. BS2 stimulated TBM degradation in the soil column experiment with different initial concentrations of 10 (a) and 15 (b) mg/kg dry soil.

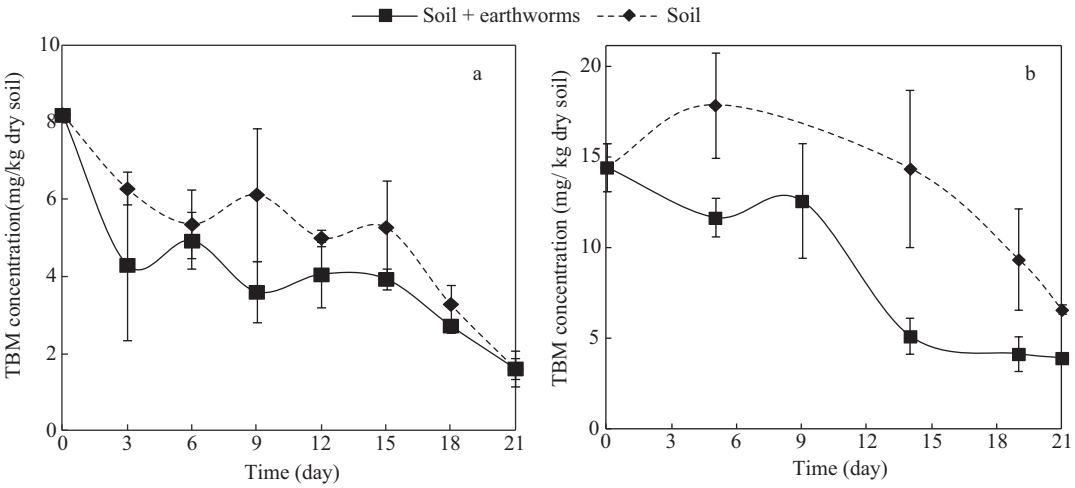

Fig. 3 Earthworm-augmented TBM degradation during soil column experiment with different initial concentrations of 10 (a) and 15 (b) mg/kg dry soil.

strain B2 (Zanardini et al., 2002), *Brevibacterium* sp. strain BH, *Methylopila* sp. strain S113 (Huang et al., 2007), *Ancylobacter* sp. XJ-412-1 (Lu et al., 2011) and *Asperigillus niger* (Boschin et al., 2003). Despite the observation that TBM biodegradation played an essential role in polluted soil (Anderson et al., 2001), few reports on TBM-degrading bacterial strains have been published in recent years. Our results suggest that diverse bacteria were involved in TBM degradation. TBM putatively served as either carbon, nitrogen or sulfur source, however, just how the bacteria were involved in TBM degradation needs further investigation. Strains BS2, BS3, and BS11 were enriched by TBM serving as sulfur source, and we found that their cell growth and degradation of TBM were different. Strain BS11 putatively assimilated TBM for growth, since it reached high cell density when TBM added in the culture broth. *Bacillus* species were frequently isolated TBM-degraders from enrichments of this study, and our results from soil column experiments demonstrated that *Bacillus* strain BS2 was potentially useful for bioremediation of TBM-polluted soil.

Previous studies showed that earthworms were able to activate indigenous soil microbial fauna and thus stimulate the mineralization of phenoxyalkanoic acid herbicide (Zaprasis et al., 2010; Liu et al., 2011). In this study, we observed that earthworms significantly stimulated TBM removal from soil columns. These observations are probably a reflection of the fact that earthworms activated indigenous TBM-degrading microbial fauna and thus stimulated TBM removal from polluted soil. We also observed that simultaneously inoculated strain BS2 and earthworms did not show a higher removal rate than either strain BS2 or earthworms alone (data not shown). The reason might be that earthworms selectively stimulated microbial populations, and strain BS2 representing a member of *Bacillus* could not be stimulated by the earthworms applied in the soil in this study.

Acknowledgments

This work was supported by the Knowledge Innovation Program of the Chinese Academy of Sciences (No. KSCX2-YW-G-052).

References

Anderson S M, Herts P B, Holst T, Bossi R, Jacobsen C S, 2001. Mineralisation studies of ^{14}C-labelled metsulfuron-methyl, tribenuron-methyl, chlorsulfuron and thifensulfuron-methyl

in one Danish soil and groundwater sediment profile. *Chemosphere*, 45(6-7): 775–782.

Beyer M E, Brown H M, Duffy M J, 1987. Sulfonylurea herbicide soil relations. In: Proceedings of the British Crop Protection Conference-Weeds. Brighton, UK. 531–540.

Boschin G, D'Agostina A, Arnoldi A, Marotta E, Zanardini E, Negri M et al., 2003. Biodegradation of chlorsulfuron and metsulfuron-methyl by *Aspergillus niger* in laboratory conditions. *Journal of Environment Science Health B*, 38(6): 737–746.

Drake H L, Horn M A, 2007. As the worm turns: The earthworm gut as a transient habitat for soil microbial biomes. *Annual Review of Microbiology*, 61(1): 169–189.

Huang X, He J, Sun J, Pan J, Li S P, 2007. Isoaltion and characterization of a metsulfuron-methyl degrading bacterium *Methylopila* sp. S113. *International Biodeterioration & Biodegradation*, 60: 152–158.

Jones C G, Lawton J H, Shachak M, 1994. Organisms as ecosystem engineers. *Oikos*, 69: 373–386.

Kaminski U, Janke D, Prauster H, Fritsche W, 1983. Degradation of aniline and monochloroanilines by *Rhodococcus* sp. An 117 and a pseudomonad: a comparative study. *Z Allg Mikrobiol*, 23(4): 235–246.

Lavelle P, 1988. Earthworm activities and the soil system. *Biology and Fertility of Soils*, 6(3): 237–251.

Liu Y J, Zaprasis A, Liu S J, Drake H L, Horn M, 2011. The Earthworm *Aporrectodea caliginosa* stimulates abundance and activity of phenoxyalkanoic acid herbicide degraders. *The ISME Journal*, 5: 473–485.

Lu P, Jin L, Liang B, Zhang J, Li S P, Feng Z Z et al., 2011. Study of biochemical pathway and enzyme involved in metsulfuron-methyl degradation by *Ancylobacter* sp. XJ-412-1 isolated from soil. *Current Microbiology*, 62(6): 1718–1725.

Ma J P, Wang Z, Lu P, Wang H J, Ali S W, Li S P et al., 2009. Biodegradation of the sulfonylurea herbicide chlorimuron-ethyl by the strain *Pseudomonas* sp. LW3. *FEMS Microbiology Letters*, 296(2): 203–209.

Ravelli A, Pantani O, Calamai L, Fusi P, 1997. Rates of chlorsulfuron degradation in three Brazilian oxisol. *Weed Research*, 37(1): 51–59.

Schaefer M, Filser J, 2007. The influence of earthworms and organic additives on the biodegradation of oil contaminated soil. *Applied Soil Ecology*, 36(1): 53–62.

Scheu S, 1987. Microbial activity and nutrient dynamics in earthworm casts (Lumbricidae). *Biology and Fertility of Soils*, 5(3): 230–234.

Si Y, Wang S, Zhou J, Hea P, Zhou D, 2005. Leaching and degradation of ethametsulfuron methyl in soil. *Chemosphere*, 60(5): 601–609.

Walker A, Cotterill E G, Welch S J, 1989. Adsorption and degradation of chlorsulfuron and metsulfuron-methy in soils from different depths. *Weed Research*, 29(4): 281–288.

Wang H, Xu J, Yates S R, Zhang J, Gan J, Ma J et al., 2010. Mineralization of metsulfuron-methyl in Chinese paddy soils. *Chemosphere*, 78(3): 335–341.

Ye Q, Sun J, Wu J, 2003. Causes of phytotoxicity of metsulfuron-methyl bound residues in soil. *Environment Pollution*, 126(3): 417–423.

Zanardini E, Arnoldi A, Boschin G, D'Agostina A, Negri M, Sorlini C, 2002. Degradation pathways of chlorsulfuron and metsulfuron-methyl by a *Pseudomonas fluorescens* strain. *Annals of Microbiology*, 52: 25–37.

Zaprasis A, Liu Y J, Liu S J, Drake H L, Horn M, 2010. Abundance of novel and diverse *tfdA*-like genes, encoding putative phenoxylakanoic acid herbicide-degrading dioxygenase, in soil. *Applied and Environmental Microbiology*, 76: 119–128.

JOURNAL OF ENVIRONMENTAL SCIENCES

Journal of Environmental Sciences (Established in 1989)
Vol. 24 No. 8 2012

Supervised by	Chinese Academy of Sciences	**Published by**	Science Press, Beijing, China
Sponsored by	Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences		Elsevier Limited, The Netherlands
		Distributed by	
Edited by	Editorial Office of Journal of Environmental Sciences (JES) P. O. Box 2871, Beijing 100085, China Tel: 86-10-62920553; http://www.jesc.ac.cn E-mail: jesc@263.net, jesc@rcees.ac.cn	Domestic	Science Press, 16 Donghuangchenggen North Street, Beijing 100717, China Local Post Offices through China
		Foreign	Elsevier Limited http://www.elsevier.com/locate/jes
Editor-in-chief	Hongxiao Tang	**Printed by**	Beijing Beilin Printing House, 100083, China

CN 11-2629/X **Domestic postcode: 2-580** **Domestic price per issue RMB ¥ 110.00**

ISSN 1001-0742