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Biodegradation of tribenuron methyl that is mediated by microbial acidohydrolysis at cell-soil interface

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ABSTRACT

Tribenuron methyl (TBM) is a member of the sulfonylurea herbicide family and is widely used in weed control. Due to its phytotoxicity to rotating-crops, concerns on TBM-pollution to soil have been raised. In this study, experimental results indicated that microbial activity played a key role in TBM removal from polluted soil. Twenty-six bacterial strains were isolated and their degradation of TBM was evaluated. *Serratia* sp. strain BW30 was selected and subjected to further investigation on its degradative mechanism. TBM degradation by strain BW30 was dependent on glucose that was converted into lactic or oxalic acids. HPLC–MS analysis revealed two end-products from TBM degradation, and they were identical to the products from TBM acidohydrolysis. Based on this observation, it is proposed that microbe-mediated acidohydrolysis of TBM was involved in TBM degradation in soil, and possible application of this observation in bioremediation of TBM-polluted soil is discussed.

1. Introduction

Tribenuron methyl (TBM) is a member of the sulfonylurea herbicide family and is widely used in weed control worldwide including in China. Although sulfonylurea herbicides are considered to be highly efficient at low dosage, their phytotoxicity to sensitive crops had 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 on wheat leads to severe reduced growth and production of rotated-crops (e.g., corn and cotton) in Henan province, due to the phytotoxicity of residual TBM in soil (Chen, 2004). Besides phytotoxicity, concerns on potential pollution to soil and groundwater by sulfonylurea herbicides or their derivatives are increasing (Si et al., 2005; Rosenbom et al., 2010). Assessment of leaching of three sulfonylurea herbicides (TBM, thifensulfuron methyl, and rimsulfuron) during irrigation indicated that thifensulfuron was leaked into groundwater (Cessna et al., 2009). Further, previous investigation showed that only 25% of TBM (initial TBM concentration was 20 μg kg^{-1}) was mineralized after 126 d in sandy soil (Anderson et al., 2001).

The residence time and fate of sulfonylurea compounds in soil are determined by many factors, including physical–chemical ones such soil acidity, temperature and moisture content, as well as biological ones such soil microbial diversity and biochemical activity (Walker et al., 1989; Ravelli et al., 1997; Wang et al., 2010). Natural hydrolysis of TBM is slowly occurring, and can be sped up by increasing acidity and light irradiation (Bottaro et al., 2008). The TBM hydrolysis proceeded via cleavage of sulfonylurea bridge, resulting in the formation of sulfonamide and triazine amine derivatives. This process has long been considered as a non-biological process and is not related to microbial activity (Sarmah and Sabadie, 2002). However, more and more researches have revealed that microbial degradation of sulfonylurea herbicides occurred via the same intermediates (Ma et al., 2009). In this study, experiments were conducted to unravel the process of TBM degradation in TBM-polluted soil and specify the role of microbial community during TBM degradation.

2. Materials and methods

2.1. Soil sampling, TBM degradation in sterilized and non-sterilized soil

Glass beakers (10 cm diameter × 14 cm height) were used to carry out soil experiments. Soil (alfisol) samples were collected from farmland and 1 kg of soil samples were dispatched into a glass beaker. The soil was autoclaved at 121 °C, 103.4 kPa for 1 h. All soil experiments were conducted in duplicate. Soil was adjusted to a final water content of 25% (determined by weight loss at 65 °C

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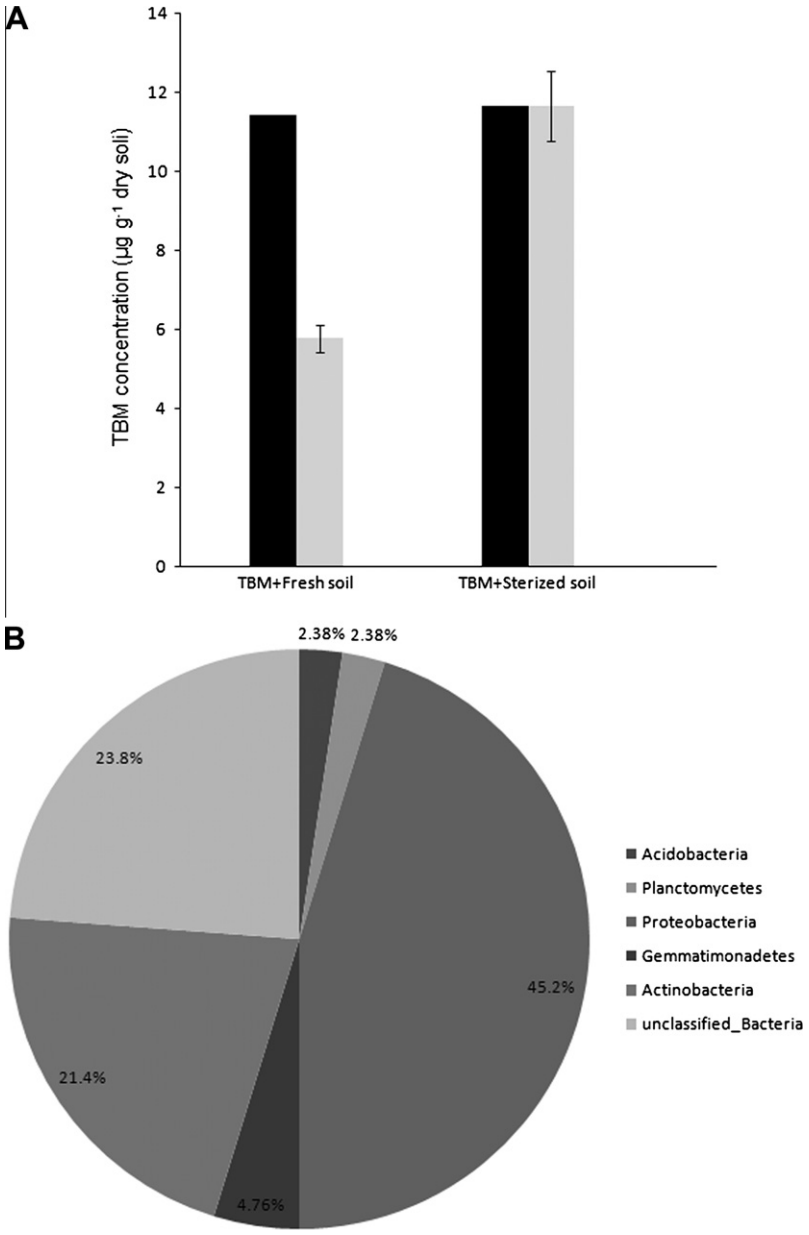

Fig. 1. Degradation of TBM in sterilized and non-sterilized soil (A) and estimation of soil microbial diversity (B). For (A), experiments were conducted in duplicates, and the data were averages of four measurements from each experiment. Black columns stand for data from day 0, and gray columns stand for data from day 4.

for 18 h) with sterile deionized water, and TBM (first dissolved in deionized water) final concentration of 10 $\mu g\, g^{-1}$ of dried soil. The soil experiments were kept in dark at room temperature. In order to monitor the TBM degradation, approximately 2 g of soil in duplicate were taken from the surfaces of each experiment to analyze the TBM concentration with HPLC.

2.2. Enrichment and isolation of TBM degrading bacteria

Soil samples used for enrichment and isolation of TBM degraders were collected from the farmland of Wuzhi, Linyin, and Yongcheng of Henan province, China, where TBM had been applied for more than 3 yr. The glucose-mineral salt (GMS) medium was composed of ($g\, L^{-1}$) glucose (2), KH_2PO_4 (0.5), $Na_2HPO_4{\cdot}12H_2O$ (1), $MgSO_4{\cdot}7H_2O$ (0.2), yeast extract (0.03), NH_4Cl (1.07), and trace elements solution (2 mL), final pH of 7.3. GMST medium was the GMS medium supplemented with TBM at a final concentration of 50 $mg\, L^{-1}$. The medium was autoclaved at 115 °C for 20 min. TBM was separately sterilized by filtration and aseptically added into the autoclaved medium.

To start the enrichment, approximately 3 g of the soil sample were added to a 250-mL Erlenmeyer flask containing 100 mL of GMST medium and incubated at 30 °C and at a rotary shaker (150 rpm) in dark. After each 7 d, 5 mL of enrichment slurry were transferred into 100 mL freshly prepared GSMT. After six transfers, the enrichment culture demonstrated strong capability of degrading TBM, and was used to isolate TBM degraders. Colonies developed on GMST agar plates were picked and isolated by repeatedly streaking on fresh GMST agar plates.

2.3. Evaluation of TBM degradation by bacterial isolates and the influences of carbon and nitrogen sources on TBM degradation

All isolates were tested for their ability to degrade TBM in 250-mL flask containing 100 mL of the GMST supplemented with TBM (50 $mg\, L^{-1}$). One isolate, named strain BW30, was subjected to fur-

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Table 1
TBM-degrading bacterial isolates from soil samples. For all tests, initial pH was adjusted to 7.0 and the initial TBM concentration was 50 mg L^{-1}. No degradation of TBM was observed in the test without bacterial inoculation. "ND" stands for "not determined", indicating the 16S rRNA gene sequence was not determined due to the strain was not cultivable.

Strains	End pH	TBM removal (%)	16S rRNA gene accession numbers at EMBL database	Closest relatives (16S rRNA gene accession numbers/identities)
BW1	5.8	75.9	ND	
BW2	5.5	100	HE578780	*Enterobacter* sp. (AB004748/99%)
BW5	5.2	80.7	ND	
BW7	5.3	100	HE578781	*Klebsiella* sp. (AF130982/100%)
BW13	5.6	100	HE578782	*Bacillus* sp. (AB021185/100%)
BW30	5.7	100	HE578779	*Serratia* sp. (AJ233427/100%)
BW33	5.6	100	HE578783	*Ochrobactrum* sp. (AY457038/100%)
BW34	5.9	100	HE578784	*Microbacterium* sp. (Y17234/99%)
BW38	4.5	100	HE578785	*Rhodococcus* sp. (X79288/99%)
BW39	5.3	100	ND	*Agromyces* sp. (X77449/98%)
BW40	3.6	100	HE578786	*Staphylococcus* sp. (HM587902/100%)
BW41	4.3	100	ND	
BW45	5.8	100	ND	
BW46	6.9	37.3	ND	
BW47	6.5	54.1	ND	
BW48	5. 6	100	ND	
BW50	6.2	100	HE578788	*Achromobacter* sp. (AJ278451/100%)
BW51	6.3	100	HE578789	*Planococcus* sp. (AJ966515/100%)
BW52	6.3	100	HE578790	*Micrococcus* sp. (FJ214355/99%)
BW53	6.2	100	HE578791	*Dietzia* sp. (AB377289/99%)
BW54	5.9	100	HE578792	*Rhodococcus* sp. (DQ090961/99%)
BY1	6.7	22.8	HE578793	*Pseudomonas* sp. (AF064458/100%)
BY3	3.9	100	ND	
BY4	7.0	59.7	HE578794	*Achromobacter* sp. (AY170847/100%)
BY6	4.8	100	HE578795	*Klebsiella* sp. (HQ259959/99%)
BY8	4.9	100	HE578796	*Raoultella* sp. (U78182/99%)

Table 2
Influences of carbon and nitrogen sources on the degradation of TBM by strain BW30. Concentrations of all carbon and nitrogen sources were set at 1 g L^{-1}. The experiments were conducted with 250-mL flasks and incubated aerobically at 150 rpm and 30 °C for 3 d. The initial concentration of TBM was 50 mg L^{-1}.

	Cell growth (OD_{600})	End pH	TBM removal (%)
Carbon sources			
Glucose	0.68	6.0	100
Glycerol	0.83	5.9	100
Sucrose	0.82	5.9	100
Starch	0.08	7.1	6.4
Sodium acetate	0.35	8.4	0
Sodium succinate	0.22	8.2	0
Sodium citrate	0.25	8.3	0
Sodium lactate	0.23	7.8	0
Sodium benzoate	0.03	7.1	0
Yeast extract	0.53	7.8	0
Peptone	0.49	7.8	0
Nitrogen sources			
NH_4Cl	0.68	6.1	100
$(NH_4)_2SO_4$	0.62	6.2	100
NH_4NO_3	0.63	6.4	88.1
Urea	0.67	7.9	2.4
Yeast extract	1.06	7.7	19.8
Peptone	1.00	7.4	65.3

ther investigation. The influences of carbon sources (glucose, glycerol, sucrose, starch, sodium acetate, sodium succinate, sodium citrate, sodium lactate, sodium benzoate, yeast extract and peptone, each at 1 g L^{-1}) and nitrogen sources (NH_4Cl, $(NH_4)_2SO_4$, NH_4NO_3, Urea, yeast extract and peptone, each at 1 g L^{-1}) on the degradation of TBM by strain BW30 were carried out at 30 °C and 150 rpm for 3 d, and cell growth (OD_{600}), final pH and TBM degradation were determined and analyzed.

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

The 16S rRNA genes of isolates and soil DNAs were amplified by polymerase chain reaction (PCR) with the universal primer pair of 27F (5′-AGAGTTTGATCCTG GCTCAG-3′) and 1492R (5′GGTTACC TTGTTACGACTT-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. All the obtained 16S rRNA gene sequences were checked for chimers and analyzed using BLASTn program, and phylogenetic analysis was performed with MEGA version 4.0.

2.5. Biotransformation of TBM with cellular lysates and cell suspension of strain BW30

Cells grown in 1 L of GMST broth at 30 °C and 150 rpm for 13 h were harvested by centrifugation at 7000*g* for 10 min. The cells obtained were washed with 20 mL of 0.13 M phosphate buffered saline (PBS) solution (pH 7.2) for three times. The obtained cells were resuspended in 9 mL of PBS buffer. Four milliliter of the cell suspension were used for biotransformation of TBM. Other 4 mL of cell suspension were subjected to ultrasonification (200 W for 7 min). Cellular lysate was obtained by removal of cell debris with centrifugation. Biotransformation of TBM with cell suspension or cellular lysate was set at 30 °C for 1 or 2 h, in the presence or absence of 1 g L^{-1} of glucose.

2.6. Identification of short-chain organic acid by HPLC

Production of short-chain organic acids by strain BW30 in GMS or GMST medium was determined with an Agilent 1200 HPLC instrument, equipped with a UV detector (wavelength at 215 nm) and a 5 μm SB reversed phase column (C_{18}, 4.6 mm id × 150 mm). The mobile phase was mixture of acetonitrile and 0.01 M KH_2PO_4 (5:95 by volume, flow rate of 0.3 mL min^{-1}, pH 2.8, adjusted with H_3PO_4). Cells were pelleted by centrifugation at 15000 *g* for 20 min, and the short-chain organic acids in the supernatants were determined. A mixture of commercial (analytical grade) oxalic acid, formic acid, pyruvic acid, lactic acid, citric acid, acetic acid, succinic acid and propionic acid was used as standard to quantify the species and concentrations of short-chain organic acids in the culture broth of strain BW30.

2.7. Identification of TBM intermediates by HPLC–MS–MS

A liquid chromatography method was adapted to analyze simultaneously TBM and its metabolites. To separate the different metabolites and the TBM molecules, an LC system (Agilent 1200

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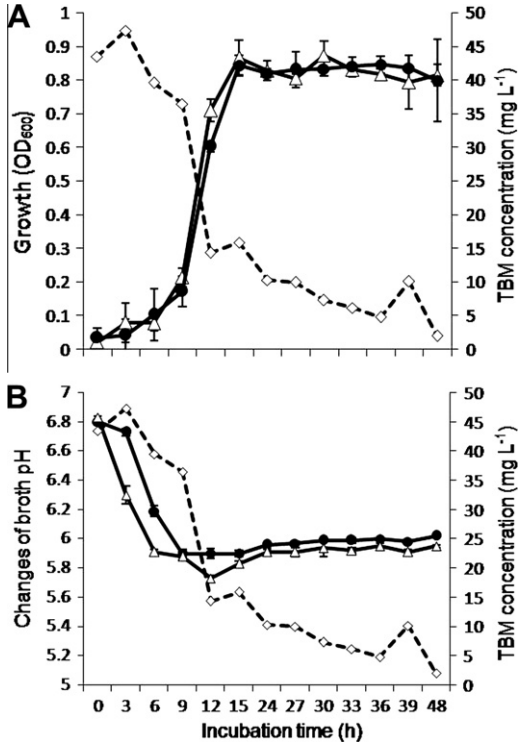

Fig. 2. Correlating cellular growth (A) and pH changes (B) to TBM degradation by strain BW30. All cultivation included 0.5 g L^{-1} of glucose. To examine TBM degradation, 50 mg L^{-1} of TBM were added in culture broth. Symbols: ●, cell growth or pH changes in the presence of TBM; △, cell growth or pH changes in the absence of TBM; Broken line stands for TBM degradation (◇).

series) equipped with a C_{18} column (4.6 mm × 250 mm × 5 μm) was used and was operated with gradient eluant of a solvent mixture (0.8 mL min^{-1}) of acetonitrile and distilled water (containing 12 μL H_3PO_4 L^{-1}). The separated molecules were detected at 225 nm with a Diode array detector on the basis of their UV spectra.

An Agilent series ion trap MS and an Agilent 1100 Series LC system were used, which equipped with a quaternary pump, an auto sampler, and a variable-wavelength detector. The MS was equipped with an electron spray ionization (ESI) source, and operated in the positive and negative polarity mode. The ESI-MS interface was operated under the conditions of a gas temperature of 350 °C and a drying gas flow of 8 L min^{-1}. The nebulizer nitrogen gas pressure is 241.3 kPa, while the capillary voltage was set to 4 kV. Full scan was obtained by scanning from 50 to 800, MS–MS experiments were carried out in product ion mode. Extend C_{18} HPLC column (5 μm, 4.6 × 150 mm) was used. The flow rate of mobile phase consisting of methanol and water (0.1% formic acid) was 1 mL min^{-1}. The initial composition was 20% acetonitrile, followed by a 20 min-linear gradient to 70% acetonitrile, then followed by a 3 min-linear gradient to 100% and hold for 5 min, and the system was re-equilibrated at the initial conditions (20% acetonitrile) from 28 to 32 min, which was held for 5 min.

2.8. Chemicals

TBM (95.8% purity) was purchased from Shanghai Pesticide Research Institute. 2-Methoxy-4-methyl-6-(methyl-amino)-1,3,5-triazine (96% purity) and methyl 2-(amino-sulfonyl)benzoate (98% purity) were purchased from Sigma–Aldrich (China).

3. Results

3.1. TBM degradation in sterilized and non-sterilized soil

The fate of sulfonylurea herbicides in soil is affected by many factors, and the microbial community is believed to be one of the most important factors. Soil samples were collected from a wheat field of Henan province that had used TBM as the main herbicides to control weed for over 3 yr. The microbial community of soil sample was estimated with 16S rRNA gene clone library, and 96 clones were randomly selected and sequenced. Results revealed that *Proteobacteria* were dominant (45%), followed by *Actinobacteria* (21%) (Fig. 1). To evaluate the contribution of microbial community to TBM degradation, TBM degradation was determined in fresh (non-autoclaved) and autoclaved soil samples. As shown in Fig. 1, fresh soil that carries diverse microbial populations removed 50% of TBM (initial concentration of TBM was 10 μg g^{-1} of dried soil) within 4 d. In contrast, the autoclaved samples did not exhibit any removal of TBM. This clearly demonstrated the involvement of microbial community in TBM degradation.

3.2. Enrichment and isolation of TBM-degrading bacteria

For the purpose to identify the microbial species and to understand the process that involved in TBM degradation, efforts were made to isolate TBM-degrading bacteria from the soil samples. TBM-degrading bacteria were enriched by consecutive supplements of TBM (50 mg L^{-1}) to soil suspension in GMS medium. After six times of transfer (6 wk), the enrichment was used to isolate TBM-degrading bacteria. In total, 26 TBM-degrading bacterial strains were isolated (Table 1). Their TBM-degrading abilities were different and the TBM removal efficiencies ranged 50–100%, as determined in our experiments. Their 16S rRNA genes were cloned and sequenced. Blast searches against GenBank database revealed these 16S rRNA gene sequences were matched to that of members of 15 bacterial genera, with sequence similarities of 97–100%. Phylogenetic analysis further revealed that these bacterial strains were new members of those 15 bacterial genera. This result indicated that TBM-degrading bacteria were quite diverse and commonly present in soil samples.

3.3. Degradation of TBM by growing cells, resting cells and cellular lysate of strain BW30

Strain BW30 (GenBank accession No. HE578779) was identified as a member of the *Serratia*, and phylogenetically identical to *Serratia marcesens* (16S rRNA gene similarity of 100%). This strain BW30 was not able to grow on TBM as sole carbon or nitrogen source. The impact of various carbon or nitrogen sources on TBM degradation is listed in Table 2. Carbon sources such as glucose, sucrose, and glycerol supported both the growth of BW30 and TBM degradation. The other tested carbon sources supported the growth of BW30 but not TBM degradation. Nitrogen sources of the ammonium salts supported both BW30 growth and TBM degradation, other nitrogen sources such as urea supported BW30 growth but not TBM degradation.

Results showed that the cell growth, pH reduction and TBM removal with strain BW30 in GMS medium was apparently correlated (Fig. 2a and b). However, cell yield in the presence or absence of 50 mg L^{-1} of TBM showed no obvious difference (Fig. 2a), possibly an indication that TBM was not assimilated into cellular materials. Further, experiments were conducted with BW30 cell suspension and cellular lysates. Results showed that TBM degradation was not dependent on the cell growth of BW30, and there was no significant difference was observed between experiments with cell suspension

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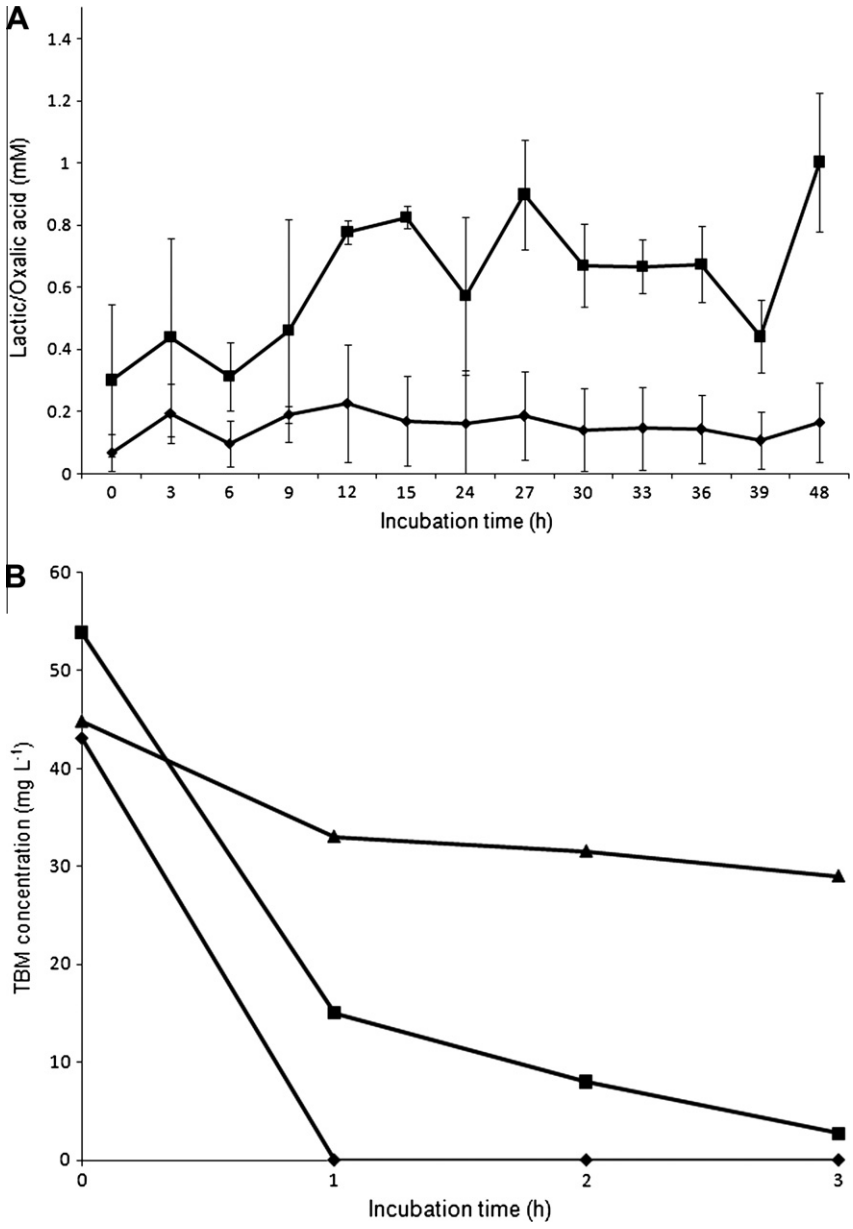

Fig. 3. Accumulation of lactic (▲) and oxalic acids (●) during growth in GSMT medium (A) and TBM acidohydrolysis (B) at various pH values in lactic and oxalic acids solution. ▲, pH 5.0; ♦, pH 6.0; △, pH 7.2.

and cellular lysate. It was found that glucose was needed for TBM degradation. When glucose was present, 13–17 and 32–40% of TBM were degraded after 1 and 2 h incubation, respectively. However, no TBM degradation was observed in the absence of glucose.

3.4. Identification of metabolites and TBM intermediates during TBM degradation by strain BW30

In attempt to understand how TBM was degraded by strain BW30, metabolites and intermediates from GMST medium were analyzed. The major metabolites accumulated during growth on GMST medium were identified as oxalic and lactic acids (Oxalic:Lactic = 4:1) (Fig. 3), which were considered to be the fermentative products of glucose. When exposed to a mixture of oxalic and lactic acids (each 0.1 mM) solution at pH of 5.0, 6.0 or 7.2, TBM was degraded quickly at lower pH (Fig. 3). Two intermediates apparently related to TBM degradation were extracted from both biodegradation and chemical degradation, and they were identified as 2-methyoxy-4-methylamino-6-methy-1,3,5-triazine and sulfonylisocyanate (Fig. 4).

4. Discussion

4.1. Microbial degradation of sulfonylurea herbicides—an observation that chemical acidohydrolysis coupled to microbial activity

Soil experiments with sterilized and non-sterilized soil were aimed to define the microbial contribution to TBM degradation. More than 50% of TBM were degraded with non-sterilized soil after 4 d, while the removal of TBM with sterilized soil was not obvious at all. These results clearly demonstrated that the soil microbial community played a major role in TBM degradation. Efforts were made to isolate TBM degrading bacteria from enrichments of these soil samples. As result, 26 strains were obtained and were identified to be members of 15 diverse genera according to their 16S

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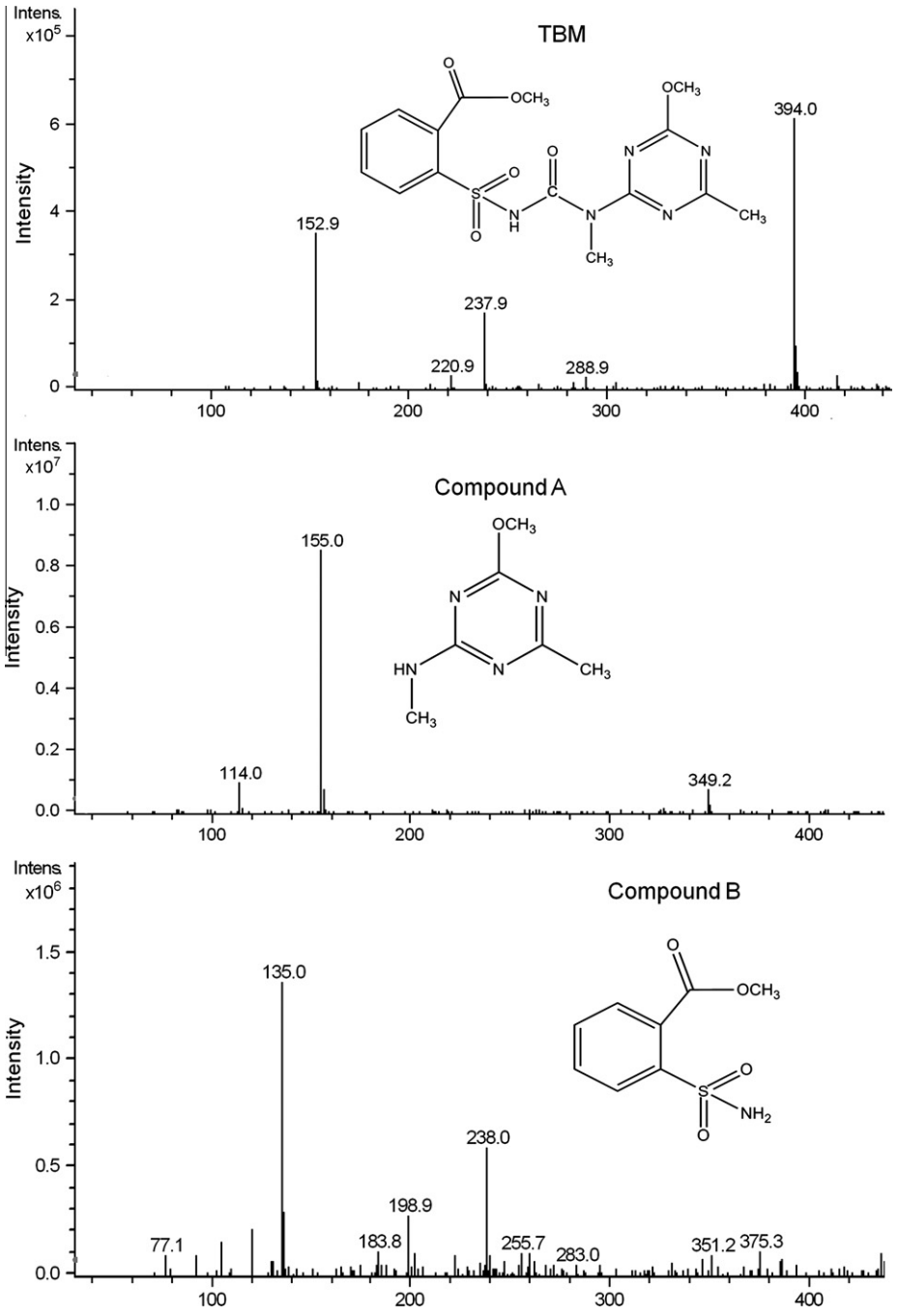

Fig. 4. Identification of intermediates with LC–MS/MS during TBM degradation.

rRNA genes. Among those genera, strain BY1 belongs to the genus *Pseudomonas*, which is well known for its robust metabolic ability and extensive involvement in degrading xenobiotic compounds. Degradation by *Pseudomonas* species was observed for TBM (Zhou NY, personal communication), metsulfuron methyl (Boschin et al., 2003), ethanmetsulfuron methyl (Gu et al., 2007), and pyrazosulfuron ethyl (Xu et al., 2009). Strain BW13 was identified as a *Bacillus* isolate, and *Bacillus* strain L1 was previously reported to be able to degrade bensulfuron methyl (Lin et al., 2010). It was previously reported that strains of *S. marcesens* were able to degraded dichlorodiphenyltrichloroethane (Bidlan and Manonmani, 2002) and hexahydro-1,3,5-trinitro-1,3,5-triazine (Young et al., 1997). Strain BW30 was phylogenetically identified as a new member of *S. marcesens*, and is characterized for its ability to degrade TBM in this study. So far as we know, bacterial isolates that degrade sulfonylurea herbicides are very limited and their degradative pathway(s) are hardly known. Thus, the finding in this study (Table 1) that diverse bacterial groups degraded TBM was really surprising.

An NADH-related carboxyesterase activity towards metsulfuron methyl was determined in cellular lysate of *Ancylobacter* strain XJ-412-1 (Lu et al., 2011). One of the TBM-degrading strains, BW30, was subjected to investigation on the TBM-degrading enzymes/pathway. Attempts on determination of any enzymatic activities with TBM as substrate, such as a putative "TBM hydrolase" activity, was failed in our study, although such a hydrolytic reaction was deducible based on the identified products (2-methyoxy-4-methylamino-6-methy-1,3,5-triazine and sulfonylisocyanate) during degradation by strain BW30. It was observed that TBM degradation was influenced by the pH value of culture broth. Therefore, we analyzed the

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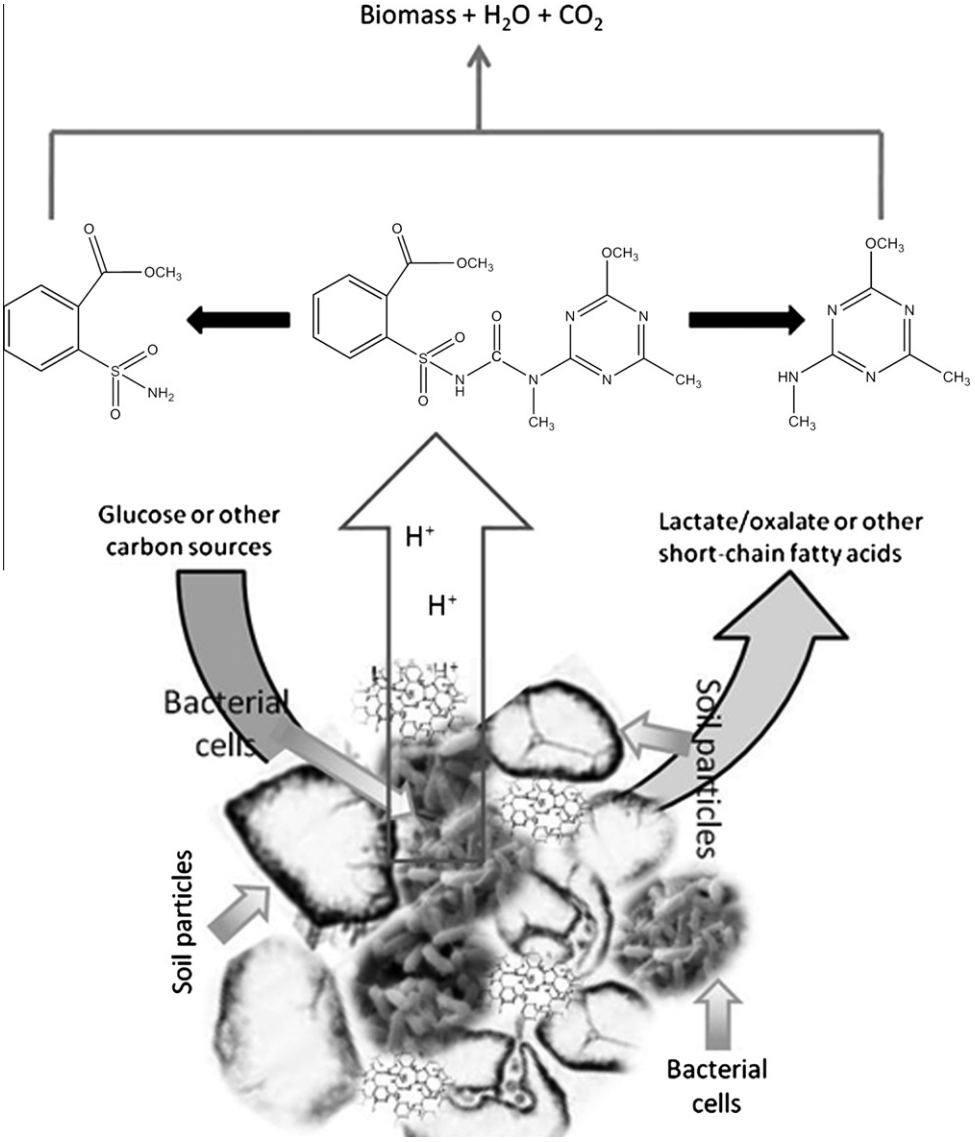

Fig. 5. Proposed microbe-mediated acidohydrolysis of TBM at microbe-soil interface.

short-chain fatty acids in BW30 culture broth, and oxalic and lactic acids were identified. In order to identify if these acids played key role in TBM degradation, experiments were carried out with oxalic and lactic acid at levels (both at 0.1 mM) similar to that of culture broth. It was found that TBM was degraded into identical products as identified from experiments with strain BW30. Based on these results, it was proposed that the degradation of TBM by strain BW30 was mediated not from a specific enzymatic catalysis but from short-chain fatty acid catalysis that were resulted from fermentation of glucose, sucrose or other carbon sources. We referred this observation as microbe-mediated acidohydrolysis of TBM.

4.2. Soil-microbe interfaces—the niches that support microbe-mediated cleavage of sulfonylurea

Acidohydrolysis was reported for a range of sulfonylurea herbicides, and it was described as an abiotic, pure chemical process (Sarmah and Sabadie, 2002; Bottaro et al., 2008). In this study, this acidohydrolysis process was apparently related to microbial activity. Strain BW30 converted glucose (or other carbon compounds in soil) into oxalic and lactic acids, the short-chain fatty acids attacked the sulfonylurea bridge and finally resulted in the breakdown of the TBM molecules (Fig. 5). Taking into account the soil experiment of which the soil bulk pH was neutral (7.2–7.5, as determined), it was further proposed that the microbe-mediated acidohydrolysis happened at micro-niches that conditioned by microbial activity. These micro-niches were physically the cell-soil interfaces, as illustrated in Fig. 5. This understanding is important for bioremediation of TBM polluted soil, particularly of land covered by neutral or alkaline soil such as the farmland in Henan province, China. With this understanding, bioremediation of TBM polluted soil would be possibly achieved by application of organic substrates that stimulate microbial activity and develop micro-niches of cell-soil for TBM degradation. Currently, such strategy is being developed in our lab and promising results are emerging.

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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, 775–782.

Beyer, M.E., Brown, H.M., Duffy, M.J., 1987. Sulfonylurea herbicide soil relations. British Crop Protection Conference – Weeds, pp. 531–540, Brighton Metropole, England.

Bidlan, R., Manonmani, H.K., 2002. Aerobic degradation of dichlorodiphen yltrichloroethane (DDT) by *Serratia marcescens* DT-1P. Process Biochem. 38, 49–56.

Boschin, G., D'Agostina, A., Arnoldi, A., Marotta, E., Zanardini, E., Negri, M., Valle, A., Sorlini, C., 2003. Biodegradation of chlorsulfuron and metsulfuron-methyl by *Aspergillus niger* in laboratory conditions. J. Environ. Sci. Health B 38, 737–746.

Bottaro, M., Frascarolo, P., Gosetti, F., Nazzucco, E., Gianotti, V., Polati, S., Piacenitni, L., Pavese, G., Gennaro, M.C., 2008. Hydrolytic and photoinduced degradation of tribenuron methyl studied by HPLC-DAD-MS/MS. J. Am. Mass Spectrom. 19, 1221–1229.

Cessna, A.J., Elliott, J.A., Bailey, J., 2009. Leaching of three sulfonylurea herbicides during sprinkler irrigation. J. Environ. Qual. 39, 365–374.

Chen, X.-L., 2004. Study on sensitivity of sulfonylurea herbicides to maize and residue dynamics. J. Hen. Vacat.-Tech. Teach. Col. 32, 24–26.

Gu, L.-F., Jiang, J.-D., Li, X.-H., Shinawar, W.A., Li, S.-P., 2007. Biodegradation of ethametsulfuron-methyl by *Pseudomonas* sp. SW4 isolated from contaminated soil. Curr. Microbiol. 55, 420–426.

Lin, X.-Y., Wang, Y., Wang, H.-L., Chirko, T., Ding, H.-T., Zhao, Y.-H., 2010. Isolation and characterization of a bensulfuron-methyl-degrading strain L1 of *Bacillus*. Pedosphere 20, 111–119.

Lu, P., Jin, L., Liang, B., Zhang, J., Li, S.P., Feng, Z.Z., Huang, X., 2011. Study of biochemical pathway and enzyme involved in metsulfuron-methyl degradation by *Ancylobacter* sp. XJ-412-1 isolated from soil. Curr. Microbiol. 62, 1718–1725.

Ma, J.-P., Wang, Z., Lu, P., Wang, H.-J., Ali, S.W., Li, S.-P., Huang, X., 2009. Biodegradation of the sulfonylurea herbicide chlorimuron-ethyl by the strain *Pseudomonas* sp. LW3. FEMS Microbiol. Lett. 296, 203–209.

Ravelli, A., Pantani, O., Calamai, L., Fusi, P., 1997. Rates of chlorsulfuron degradation in three Brazilian noxisol. Weed Res. 37, 51–59.

Rosenbom, A.E., Kjaer, J., Olsen, P., 2010. Long-term leaching of rimsulfuron degradation products through sandy agricultural soils. Chemosphere 79, 830–838.

Sarmah, A.K., Sabadie, A.J., 2002. Hydrolysis of sulfonylurea herbicides in soils and aqueous solutions: a review. J. Agri. Food Chem. 50, 6253–6265.

Si, Y., Wang, S., Zhou, J., Hua, R., Zhou, D., 2005. Leaching and degradation of ethametsulfuron-methyl in soil. Chemosphere 60, 601–609.

Walker, A., Cotterill, E.G., Welch, S.J., 1989. Adsorption and degradation of chlorsulfuron and metsulfuron-methy in soil from different depths. Weed Res. 29, 281–288.

Wang, H., Xu, J., Yates, S.R., Zhang, J., Gan, J., Ma, J., Wu, J., Xuan, R., 2010. Mineralization of metsulfuron-methyl in Chinese paddy soils. Chemosphere 78, 335–341.

Xu, J., Li, X.-S., Xu, Y.-J., Qiu, L.-H., Pan, C.-P., 2009. Biodegradation of pyrazosulfuron-ethyl by three strains of bacteria isolated from contaminated soils. Chemosphere 74, 682–687.

Ye, Q., Sun, J., Wu, J., 2003. Causes of phytotoxicity of metsulfuron-methyl bound residues in soil. Environ. Pollut. 126, 417–423.

Young, D.M., Unkefer, P.J., Ogden, K.L., 1997. Biotransformation of hexahydro-1,3,5-trinitro-1,3,5-trazine (RDX) by a prospective consortium and its most effective isolate *Serratia* marcescens. Biotechnol. Bioeng. 53, 515–522.

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