Science of the Total Environment 472 (2014) 502–508

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Dynamics of communities of bacteria and ammonia-oxidizing microorganisms in response to simazine attenuation in agricultural soil

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HIGHLIGHTS

- Autochthonous microbial community in agricultural soil had a strong self-remediation potential of simazine pollution.
- Simazine amendment could alter the community structures of total bacteria and ammonia-oxidizing archaea and bacteria.
- A shift in the composition of major bacterial groups occurred with simazine biodegradation.
- A variety of microorganisms might play a role in simazine biodegradation.

ARTICLE INFO

Article history:
Received 28 September 2013
Received in revised form 11 November 2013
Accepted 18 November 2013
Available online 6 December 2013

Keywords:
Ammonia-oxidizing bacteria (AOB)
Ammonia-oxidizing archaea (AOA)
Arthrobacter
Biodegradation
s-triazine herbicides
Microbial community

ABSTRACT

Autochthonous microbiota plays a crucial role in natural attenuation of *s*-triazine herbicides in agricultural soil. Soil microcosm study was carried out to investigate the shift in the structures of soil autochthonous microbial communities and the potential degraders associated with natural simazine attenuation. The relative abundance of soil autochthonous degraders and the structures of microbial communities were assessed using quantitative PCR (q-PCR) and terminal restriction fragment length polymorphism (TRFLP), respectively. Phylogenetic composition of bacterial community was also characterized using clone library analysis. Soil autochthonous microbiota could almost completely clean up simazine (100 mg kg^{-1}) in 10 days after herbicide application, indicating a strong self-remediation potential of agricultural soil. A significant increase in the proportion of *s*-triazine-degrading *atzC* gene was found in 6 days after simazine amendment. Simazine application could alter the community structures of total bacteria and ammonia-oxidizing archaea (AOA) and bacteria (AOB). AOA were more responsive to simazine application compared to AOB and bacteria. *Actinobacteria*, *Alphaproteobacteria* and *Gammaproteobacteria* were the dominant bacterial groups either at the initial stage after simazine amendment or at the end stage of herbicide biodegradation, but *Actinobacteria* predominated at the middle stage of biodegradation. Microorganisms from several bacterial genera might be involved in simazine biodegradation. This work could add some new insights on the bioremediation of herbicides contaminated agricultural soils.

1. Introduction

The widespread use of simazine and other *s*-triazine herbicides for control of grass and broad-leaved weeds together with their persistence in the environment has aroused increasing environmental concern (Fajardo et al., 2012; Grenni et al., 2012; Morgante et al., 2012). Repeated application of *s*-triazine herbicides can result in the adaptation of soil bacterial community capable of rapidly degrading these compounds (Getenga et al., 2009; Krutz et al., 2008; Zablotowicz et al., 2006). Microbial degradation is mainly responsible for natural *s*-triazine attenuation in agricultural soil with a long history of herbicide application (Fajardo et al., 2012; Getenga et al., 2009; Martin et al., 2008; Wang and Xie, 2012). To date, a variety of *s*-triazine-degrading microorganisms from diverse genera have been isolated and characterized (Getenga et al., 2009), but few of them show high capacity to mineralize the *s*-triazine ring (Fajardo et al., 2012). *Pseudomonas* sp. ADP is the most known and the best-characterized *s*-triazine-degrading bacterium, yet microorganisms from genus *Arthrobacter* are known for their strong capacity to degrade *s*-triazine herbicides (Guo et al., 2013; Strong et al., 2002; Zhou et al., 2013).

However, most of microorganisms in the environment are usually hard to be isolated, and different culture conditions may select for the specific *s*-triazine degraders. There is still a wide variety of uncultivated *s*-triazine-degrading bacteria in soil ecosystems (Xie et al., 2013).

Culture independent approaches would be more suitable for identification of the *s*-triazine-degrading population in soil ecosystems. Fingerprinting techniques (e.g., denaturing gradient gel electrophoresis (DGGE), ribosomal intergenic spacer analysis (RISA), or terminal restriction fragment length polymorphism (TRFLP)) have commonly

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http://dx.doi.org/10.1016/j.scitotenv.2013.11.090

been applied to characterize the *s*-triazine-degrading bacterial community in bioaugmented or non-bioaugmented soils (Briceno et al., 2010; Guo et al., 2013; Martin-Laurent et al., 2006; Morán et al., 2006; Xie et al., 2013; Zhou et al., 2013). Fluorescence in situ hybridization (FISH) technique has also been used for direct recognition of the target indigenous *s*-triazine-degraders in agricultural soils (Fajardo et al., 2012; Jose et al., 2010; Martin et al., 2008). To date, very limited information is available on the phylogenetic composition of soil indigenous *s*-triazine-degrading population. The change of microbial groups associated with natural attenuation of herbicides remains unclear (Morgante et al., 2010). Moreover, quantitative PCR assay has widely used to estimate the *s*-triazine-degrading genetic potential of soil indigenous bacterial community (Jose et al., 2010; Martin-Laurent et al., 2004; Piutti et al., 2002), or monitor the survival of added *s*-triazine degraders in soils (Guo et al., 2013; Xie et al., 2013; Zhou et al., 2013). However, little is known about the shift in the abundance of autochthonous degraders in response to natural attenuation of *s*-triazine herbicides in agricultural soil. Therefore, the potential and function of soil autochthonous bacterial community associated with biodegradation of *s*-triazine herbicides remain unclear.

Ammonia oxidation is an important process for global nitrogen cycling. Both ammonia-oxidizing archaea (AOA) and bacteria (AOB) have been widely accepted as the important players in soil nitrification process, although their relative contribution to nitrification remains controversial (Wang et al., 2013). Soil AOA and AOB community structures can be affected by many environmental factors (Wang et al., 2013). Nitrification is considered to be a relatively sensitive indicator of the ecotoxicological effects of agrochemicals on soil microorganisms (Malkomes, 1992). Pesticide application can usually inhibit nitrification process in agricultural soil (Pell et al., 1998). However, to date, only few previous studies have investigated the impacts of pesticides on soil AOA and AOB community structures (Guo et al., 2013; Hernandez et al., 2011; Li et al., 2008; Puglisi et al., 2012). The selective pressure on soil AOA and AOB communities may be alleviated with biodegradation of *s*-triazine herbicides, which can result in a shift of their structures. Information on the shift of AOA and AOB community structures in response to natural attenuation of *s*-triazine herbicides is still lacking.

The aim of the current study was to carry out an investigation on the shift in the structures of autochthonous microbial communities in response to natural simazine attenuation in agricultural soil. The shifts in community structures of bacteria, AOA and AOB were assessed using TRFLP analysis. *atzC* gene is one of the known *s*-triazine-degrading genes and is found in phylogenetically diverse bacteria. The abundance of soil autochthonous degraders was monitored using quantitative PCR assay targeting at *atzC* gene. Moreover, clone library analysis was used for further phylogenetic identification of bacterial community composition.

2. Materials and methods

2.1. Microcosm set-up

Soil was collected from a farmland that has received typical simazine application (about 4–5 mg kg^{-1}) for more than 20 years. At time of sample collection, no residual simazine in soil was detected. The soil was slightly alkaline (pH 8.2) and loam, with ammonia nitrogen content of 9.1 mg kg^{-1} and carbon content of 15.5 g kg^{-1}. Soil microcosms were prepared in 250-mL jars with 50 g soil (dry weight), and then incubated at 25 °C in the dark. For sterile control experiment, soil was autoclaved (121 °C, 30 min) in three different days. Soil moisture was maintained at 10–15% of the water-holding capacity. Three sets of treatments in triplicate were performed as follows: (A) soil + 100 mg kg^{-1} simazine; (B) soil (as blank control); and (C) sterilized soil + 100 mg kg^{-1} simazine (as sterile control).

2.2. Chemical and molecular analyses

For chemical analysis of residual simazine in soil, soil samples (1 g, dry weight) from the treatments A and C were collected at days 0, 3, 6 and 10. The residual simazine in soil was extracted and analyzed as previously described (Guo et al., 2013). Briefly, soil sample was extracted with methanol under a 30-min ultrasonication, followed by a 5-min centrifugation. The simazine concentration was then determined by high performance liquid chromatography (HPLC) analysis, using methanol–water (70:30) as the mobile phase at a flow rate of 1.0 mL min^{-1}. The recovery rate of simazine extraction was 97–99%.

For molecular analysis, soil samples (0.5 g, dry weight) from the treatments A and B were collected at days 0, 6, 10, 19 and 29. Soil DNA samples were extracted using the Powersoil DNA extraction kit (Mobio Laboratories). DNA concentration was quantified using Nanodrop® ND-1000 UV–vis spectrophotometry (USA). The specific primers for amplification of the *ammonia monooxygenase A* (*amoA*) genes of AOA and AOB were Arch-*amoA*F (5′-STAATGGTCTGGCTTA GACG-3′; 5′ end-labeled with carboxyfluorescine), Arch-*amoA*R (5′-GCGGCCATCCATCTGTATGT-3′), *amoA*-1 F (5′-GGGGTTTCTACTGGTGGT-3′; 5′ end-labeled with carboxyfluorescine), and *amoA*-2R (5′-CCCCTC KGSAAAGCCTTCTTC-3′) (Feng et al., 2012). The PCR reactions were also performed according to the literature (Feng et al., 2012). Soil bacterial 16S rRNA were amplified using 27 F-FAM (5′-AGAGTTTG ATCMTGGCTCAG-3′, 5′ end labeled with carboxyfluorescine), and 1492R (5′-GGTTACCTTGTTACGACTT-3′), using the same PCR conditions as previously described (Xie et al., 2013; Zhang et al., 2012a,b). Purified PCR products (300 ng) were digested with *Hha*I. TRFLP fragments were analyzed using an ABI 3730 DNA Analyzer (Applied Biosystems) and Genemapper v3.5 software (Applied Biosystems). Clustering of samples was performed using software PRIMER 5.0 and the UPGMA (unweighted pair group mean average) method (Clarke and Warwick, 2001).

For construction of each bacterial clone library, DNA from triplicate samples was pooled. The PCR conditions were the same as the above-mentioned, except the forward primer was unlabeled (27 F 5′-AGAG TTTGATCMTGGCTCAG-3′). Clone libraries were constructed according to the literatures (Zhang et al., 2012b,c). PCR products were cloned into pMD19-T vector (Takara Corp, Japan) with ampicillin selection and blue/white screening. The clones containing correct size were sequenced. Chimera-free bacterial sequences with ≥97% identity were assigned as an operational taxonomic unit (OTU) using the DOTUR program (Schloss and Handelsman, 2005). OTU-based Shannon diversity index were also obtained using the DOTUR program (Schloss and Handelsman, 2005). The Ribosomal Database Project (Center for Microbial Ecology, Michigan State University) analysis tool "classifier" was utilized to classify the taxonomic identity of bacterial sequence (Wang et al., 2007). The bacterial sequences obtained in this study were submitted to GenBank, under accession numbers KF290774–KF290955.

For quantitative PCR assays, the specific primers for amplification of the 16S rRNA and *s*-triazine-degrading *atzC* genes and the PCR conditions were the same as previously described (Guo et al., 2013; Zhou et al., 2013), 16Sf (5′-CTGGTAGTCCACGCCGTAAA-3′), 16Sr (5′-CGAATTAAACCACATGCTCCAC-3′), atzCf (5′-GCTCACATGCAGGT ACTCCA-3′), and atzCr (5′-TCCCCCAACTAAATCACAGC-3′). The amplification efficiency and coefficient ($r2$) for amplification of the 16S rRNA and *atzC* genes were 102% and 97%, 0.998 and 0.999, respectively. Ratio of *atzC* gene copy to 16S rRNA gene copy was used to estimate the relative abundance of the autochthonous simazine degraders in soil microcosms (Guo et al., 2013).

Analysis of variance (two-way analysis of variance) was used to determine the significant differences ($p < 0.05$) in the residual simazine rate and the relative abundance of *atzC* gene among the different treatments during the incubation period, using the statistical software SPSS 20.