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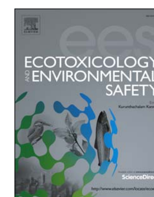

Efficient degradation of chlorimuron-ethyl by a bacterial consortium and shifts in the aboriginal microorganism community during the bioremediation of contaminated-soil

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ABSTRACT

Excessive application of chlorimuron-ethyl has led to soil contamination and limited crop rotation; therefore, tactics to decrease and eliminate residual chlorimuron-ethyl in the environment have attracted increasing attention. In this study, two chlorimuron-ethyl-degrading bacterial strains (*Rhodococcus* sp. D310-1; *Enterobacter* sp. D310-5) were used to ferment and prepare a chlorimuron-ethyl-degrading bacterial consortium. To improve the degradation efficiency of the bacterial consortium, the cultivation conditions were optimized using response surface methodology (RSM). The maximum biodegradation rate (87.42%) was obtained under optimal conditions (carbon concentration, 9.21 g L^{-1}; temperature, 26.15 °C; pH, 6.95). The rate of chlorimuron-ethyl degradation by the bacterial consortium in the chlorimuron-ethyl-contaminated soil was monitored and reached 80.02% at the end of a 60-d incubation period. Illumina MiSeq sequencing results showed that microbial diversity was high, and 33 phyla were identified in the analyzed samples. Proteobacteria, Acidobacteria, Acidobacteria, Firmicutes and Bacteroidetes were present in relatively high abundances in the samples. The bacterial consortium made a positive impact on the remediation of chlorimuron-ethyl-contaminated soil and somewhat altered the composition of the bacterial community in the chlorimuron-ethyl-contaminated soil. These findings provide highly valuable information on the production of bacterial consortium for the remediation of chlorimuron-ethyl and other sulfonylurea-herbicide-contaminated soil.

1. Introduction

Chlorimuron-ethyl [ethyl 2-(((((4-chloro-6-methoxypyrimidin-2-yl) amino) carbonyl) amino) sulfonyl) benzoate] is a long-term residual sulfonylurea herbicide. Because of its low mammalian toxicity, chlorimuron-ethyl has been widely used in modern agriculture for weed control (Tan et al., 2013; Zhao and He et al., 2007). In Heilongjiang Province alone, 400 t of chlorimuron-ethyl is used annually in soybean fields to control broadleaf weeds (Zhao and He, 2007). However, the long-term and excessive application of chlorimuron-ethyl, even at low concentrations, has limited crop rotation and affected the soil microbial community and soil enzymes (Zhang et al., 2009; Tan et al., 2013). Additionally, chlorimuron-ethyl easily leaches from the soil and contaminates the groundwater due to its low k_{ow} value and high water solubility (Tan et al., 2013). Therefore, remediation of chlorimuron-ethyl-polluted soil is a crucial environmental issue.

Some studies have aimed to reduce and eliminate residual chlorimuron-ethyl, including the use of physical, chemical and biological methods (Zhang et al., 2009). Bioaugmentation is a relatively cost-effective and environmentally friendly method that has received considerable attention (Al-Kharusi et al., 2016). Many microbes that degrade chlorimuron-ethyl have been isolated and characterized, such as *Pseudomonas* sp. LW3 (Ma et al., 2009), *Klebsiella jilinsis* 2N3 (Zhang et al., 2010), *Rhodococcus* sp. D310-1 (Xiong et al., 2011), *Stenotrophomonas maltophilia* (Li et al., 2011), *Aspergillus niger* (Sharma et al., 2012) and *Hansschlegelia* sp. CHL1 (Yang et al., 2014). Nevertheless, most studies have screen and characterized chlorimuron-ethyl-degrading microorganisms and assessed the degradation rate of these organisms (Zhang et al., 2009). Only a few reports have focused on the effectiveness of the remediation of chlorimuron-ethyl-contaminated soil by the inoculation of chlorimuron-ethyl-degrading microorganisms. For example, Yang et al. (2014) used the chlorimuron-ethyl degrading strain CHL1 for soil bioremediation and studied the survival time of the degrading strain and changes in the abundance of bacteria, fungi and N-cycling functional genes during bioremediation. Unfortunately, few studies have investigated changes in the generic

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structure of the bacterial community after the application of a bacterial consortium to chlorimuron-ethyl-contaminated soil.

The principal barrier to bioaugmentation is the inability of the introduced microorganisms to cope with the harsh environmental conditions in the field (Al-Kharusi et al., 2016). Additionally, degrading bacteria do not survive long in liquid medium, which is easily contaminated, and the conservation effect of bacterium in liquid medium is dissatisfactory (Jiang et al., 2008). Therefore, the development of a solid carrier that can absorb a large quantity of live bacteria and provide the inoculated bacteria with a protective surface or pore space and create a protective microhabitat is a key factor in the successful remediation of chlorimuron-ethyl-contaminated soil (Jiang et al., 2008; Yang et al., 2014). To the best of our knowledge, little detailed research has been conducted on the preparation of a chlorimuron-ethyl-degrading bacterial consortium and its application for the bioremediation of chlorimuron-ethyl-contaminated soil.

In this study, we used a mixed culture of the highly efficient chlorimuron-ethyl-degrading bacteria *Rhodococcus* sp. D310-1 (Xiong et al., 2011) and *Enterobacter* sp. D310-5 (Li et al., 2016a), both of which were isolated from sewage sludge samples collected from the activated sludge tank of a chlorimuron-ethyl production factory in China, to ferment and prepare a chlorimuron-ethyl-degrading bacterial consortium. The fermentation medium, mixed culture conditions and preparation protocol for the bacterial consortium were optimized by response surface methodology (RSM) to improve the degradation rate. In addition, the bacterial consortium was used to remediate chlorimuron-ethyl-contaminated soil. Changes in the composition of the bacterial community in the soil were evaluated using Illumina MiSeq sequencing. The data from this study will contribute considerably to optimizing the remediation of chlorimuron-ethyl-contaminated soil and will provide information for the assessment of the bacterial community in the soil, including nonculturable organisms and those with a low abundance, and for other attempts at the bioremediation of sulfonylurea-herbicide-contaminated soil.

2. Materials and methods

2.1. Bacterial strains

Rhodococcus sp. D310-1 and *Enterobacter* sp. D310-5 were isolated from factories producing sulfonylurea herbicides in China and stored in our laboratory (detailed information is provided as Supplementary material).

2.2. Chemicals and media

Chlorimuron-ethyl (purity 98.70%) was purchased from the Jiangsu Institute of Ecomones Co., Ltd., China. All chemicals used in this study were of analytical reagent grade and obtained from Tianjin Kemiou Chemical Reagent Co., Ltd.

The composition of the mineral salt medium (MSM) was as follows (L^{-1}): $CaSO_4$ 40 mg, K_2HPO_4 100 mg, NaCl 100 mg, $FeSO_4 \cdot 7H_2O$ 1 mg, $MgSO_4 \cdot 7H_2O$ 200 mg and $(NH_4)_2SO_4$ 100 mg, pH 6.0-6.

2.3. Soil sampling

Soil samples were collected at the Horticultural Research Station of Northeast Agricultural University (Harbin, China) from an area of abandoned land that had been free of herbicides and fertilizer for 16 years. The soil samples were sieved through a 2-mm mesh screen for experimental treatment after transport to the laboratory. The soil characteristics (pH; total N, P, and K; and water-holding capacity) were determined.

The physical and chemical properties of the soil were as follows: total nitrogen (N) 93.5 mg kg^{-1}, total phosphorus (P) 42.1 mg kg^{-1}, total potassium (K) 416.9 mg kg^{-1}, water-holding capacity 2.9%, and pH 6.23.

2.4. Optimization of the conditions for preparing the chlorimuron-ethyl-degrading bacterial consortium

The growth interactions between strain D310-1 and D310-5 and the effects of carbon sources on the growth of mixed bacteria and chlorimuron-ethyl degradation were studied. The effects of chlorimuron-ethyl concentrations on mixed bacteria in fermentation medium were determined, and the growth and degradation conditions of mixed bacteria were optimized. The selection of microbial carriers and the optimal conditions for preparing a chlorimuron-ethyl-degrading bacterial consortium were studied. The degradation conditions of the bacterial consortium were optimized (detailed experimental methods are provided as Supplementary material). All of the samples were processed, and the degradation rates were determined as described in Sections 2.6 and 2.7.

2.5. Remediation of chlorimuron-ethyl-contaminated soil by a bacterial consortium

2.5.1. Optimization of the dosage of the bacterial consortium added to the soil

The dosage of the bacterial consortium added to the soil was optimized in a greenhouse. The soil was collected and prepared as described in Section 2.3. Chlorimuron-ethyl (20 mg kg^{-1}) was added to natural soil (NS). Different dosages of the bacterial consortium (0.1 g kg^{-1}, 0.5 g kg^{-1}, 1.0 g kg^{-1}, 1.5 g kg^{-1} and 2.0 g kg^{-1}) were added to NS contaminated with chlorimuron-ethyl. All carriers were mixed with the fermentation liquid, cultured at 33 °C and dried at 36 °C to obtain the bacterial consortium. The chlorimuron-ethyl degradation rate was determined by HPLC after 30 d as described in Section 2.7. All of the treatments were replicated three times to minimize experimental error.

2.5.2. Remediation of chlorimuron-ethyl-contaminated soil

Based on Section 2.5.1, three treatments using the optimized dosage determined as previously outlined were set up to test the remediation of the chlorimuron-ethyl-contaminated soil: NS, NS contaminated with 20 mg kg^{-1} chlorimuron-ethyl (NS-CE), and NS-CE inoculated with the bacterial consortium (NS-CE-B).

Soil samples were collected at 5, 10, 15, 20, 30, 40, 50, 55 and 60 d, and the residual chlorimuron-ethyl was determined. The effects of the bacterial consortium on the indigenous bacterial community were evaluated in the chlorimuron-ethyl-contaminated soil using Illumina MiSeq sequencing.

2.5.3. Illumina MiSeq sequencing of 16 S rRNA genes: bacterial diversity and relative abundance in the soil samples

DNA was extracted from the soil following the protocol of Graham et al. (2000) and purified using an EasyPure® Quick Gel Extraction Kit (TransGen Biotech, Beijing, China) according to the manufacturer's instructions. The total DNA concentration was quantified using a Qubit Fluorometer. The V3-V4 region of the 16 S rRNA gene was amplified by PCR with the universal prokaryotic primer set: 515 F: GTGCCAGCMGCCGCGGTAA; 806 R: GGACTACHVGGGTWTCTAAT. The amplification conditions were 5 min at 94 °C; 35 cycles of 1 min at 94 °C, 1 min at 62 °C and 1 min at 72 °C; and 10 min at 72 °C.

2.5.4. Quality filtering of reads, OTU selection, and taxonomic assignments

To obtain more accurate and reliable results in the subsequent bioinformatics analysis, the raw data were pre-processed to obtain clean data. 1) Sequence reads that had an average quality of less than 20 over a 10-bp sliding window were truncated based on the phred algorithm. Trimmed reads that had less than 75% of their original length were removed, along with the paired reads. 2) Reads contami-

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